

Water Microbiology

MICHIGAN STATE
UNIVERSITY

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Our group works in four areas on water quality that are interconnected.

- **New Methods for microbial detection (particularly molecular methods)**
- **Survey of pathogens and indicators in water**
- **Evaluation of treatment technology**
- **Quantitative microbial risk assessment**

SAFE DRINKING WATER ACT

- National Policy for safe drinking water. Health focus: MCL, treatment regulations
- Total Coliform Rule
- Long-Term Enhanced Surface Water Treatment Rule: *Viruses, Cryptosporidium and Giardia*
- Information Collection Rule
- Ground Water Rule
- Unregulated Contaminate Monitoring Rule
- Contaminant Candidate List
- Consumer Confidence Reports
- Watershed and Groundwater Protection
- Sensitive Populations

THE U.S. WATER CHALLENGES

- Climate Variability: floods and droughts. Prediction of Global Warming, sea level, changes in precipitation.
- Infrastructure: EPA Gap report suggests 52 billion dollars needed in the next decade.
- New Technology: Detection Technology New Contaminants at Lower levels. Treatment Technology, Membranes and UV disinfection.
- Sensitive Populations: Immunocompromised and Elderly, 30% of our communities.
- Home Land Security: Water Vulnerability.

Water Quality & Health

Threats

- Fecal contamination
- Sewage
- Septic tanks
- Combined sewer overflows
- Stormwater runoff
- Inadequate infrastructure
- Toxic Algal blooms
- Climate change

Consequences

- Waterborne disease
- Boil orders
- Community Outbreaks
- Acute and chronic affects



What to monitor?

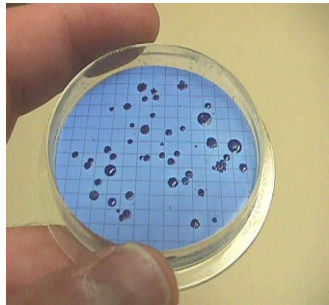
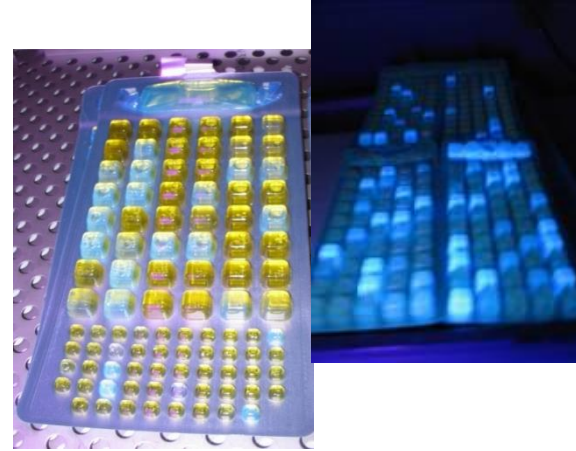
Indicators

- Related to fecal pollution
- Higher concentrations = easier to sample
- Less expensive
- Examples: *E. coli*, total coliform bacteria

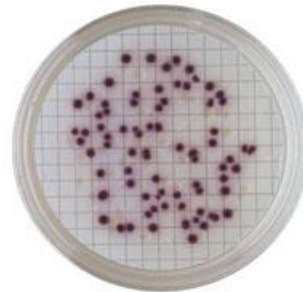
Pathogens

- Cause disease
- Low concentrations = more challenging
- More expensive
- Examples: *Salmonella*, *Adenovirus*, norovirus, *Cryptosporidium* and *Giardia*.

Growth Based Methods: Common Fecal Indicator Organisms



Fecal
coliforms



E.coli

Total
coliforms

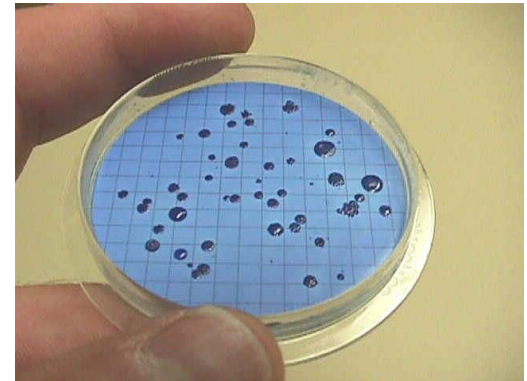
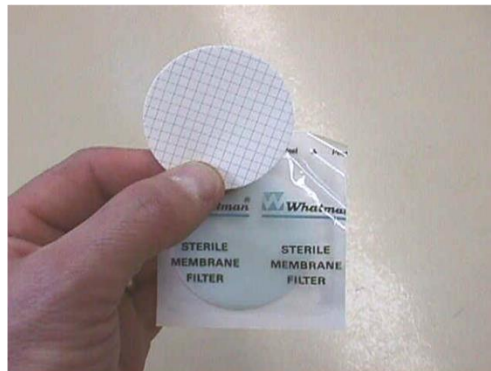


Enterococci

All bacteria, non-sporeformers

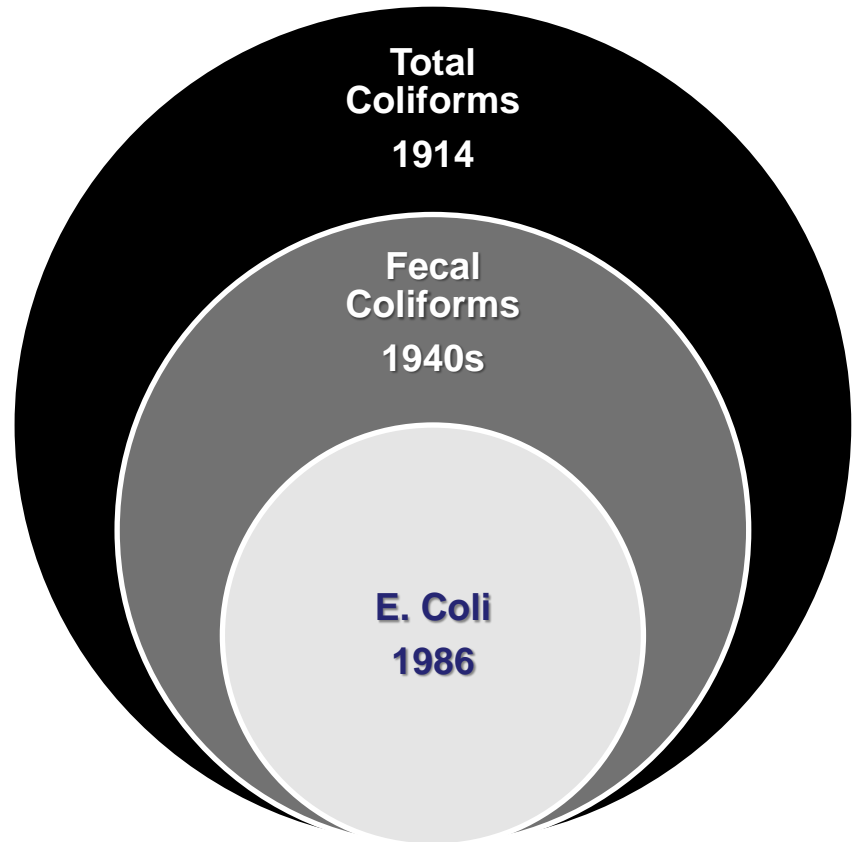
Assessing Water Quality

- Monitoring by utilities, water labs, local health departments, states, federal governments, academic scientists
- Use reliable, trusted techniques for detecting fecal contamination
- Current standards based on *E. coli*



Evolution of Monitoring

- Methods changed over time as technology advanced



GLOBAL Universal Indicator for drinking water $E.coli < 1/100ml$

Innovative Water Technology

Water Genomics and Safety

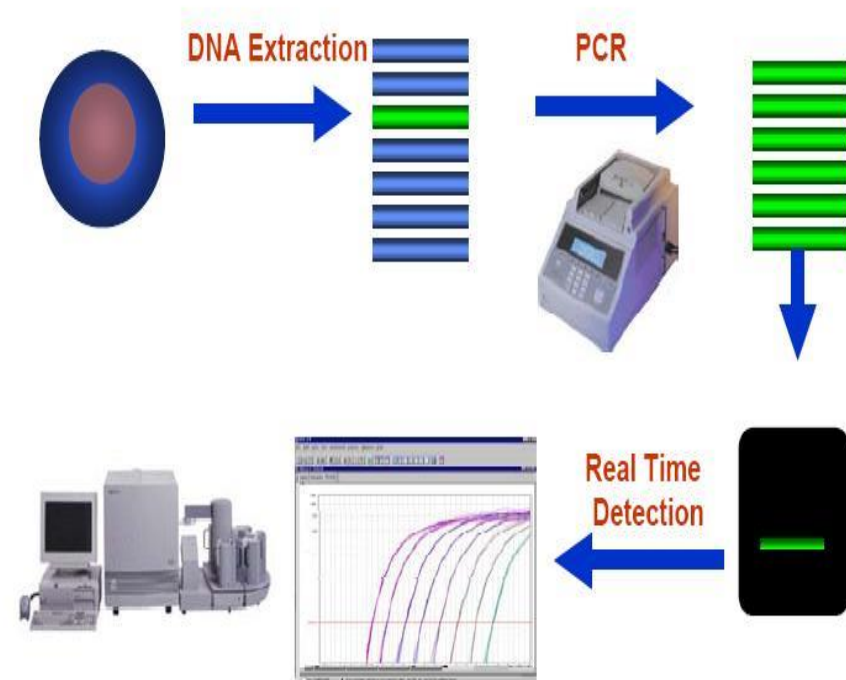
Polymerase chain reaction (PCR):

Small amount of DNA amplified in a thermal cycler

Amplified products are measured at the end point of amplification by agarose gel electrophoresis

Quantitative PCR (qPCR):

Amplified PCR products are detected real-time during the early phases of the reaction.



Polymerase Chain Reaction (PCR)

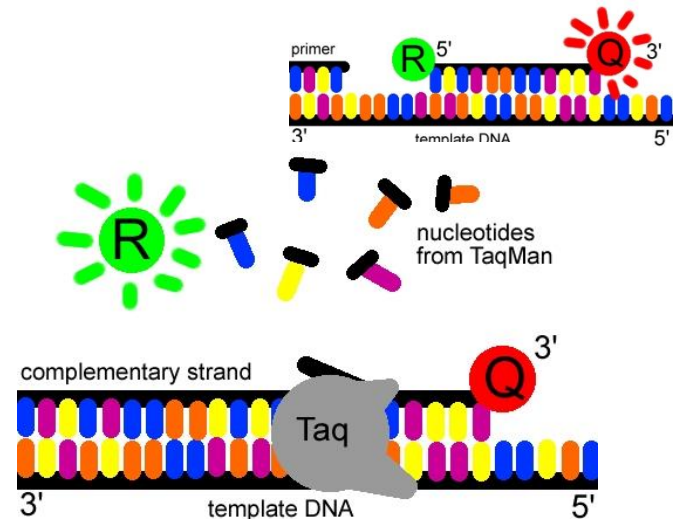
- **Developed in 1985 by Kary Mullis**
- **Dr. Mullis received the Nobel Prize in Chemistry in 1993**
- **PCR is considered as one of the most important discoveries in molecular biology**

What is PCR?

- **Enzymatic reaction that makes many copies of DNA from single molecule**
- **2^n copies of DNA from single molecule where $n = \text{No. of cycles}$**
- **So, 35 cycles of PCR would yield 2^{35} copies of DNA**

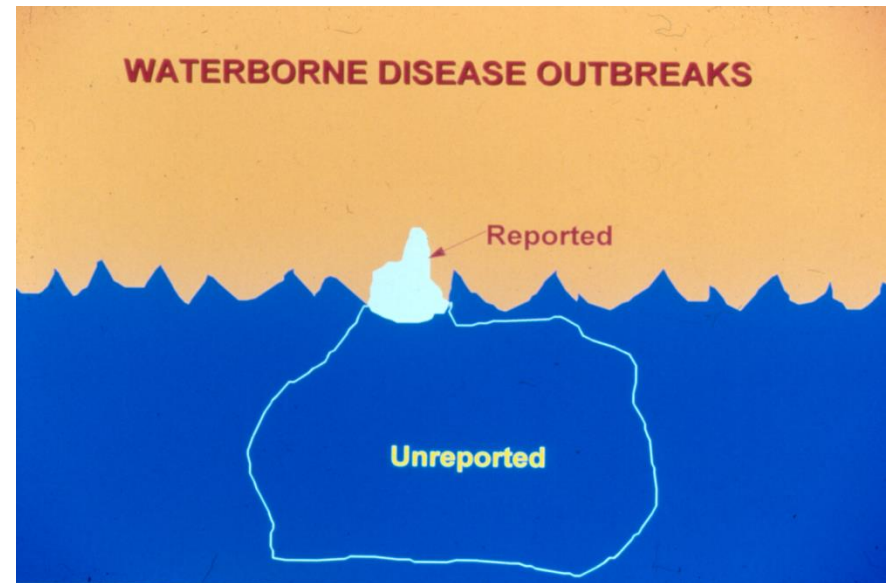
REAL TIME qPCR method For BEACH Monitoring

- A new standard for water monitoring has been approved by the EPA to use qPCR for Enterococci.
- Also PCR plus cell culture approved for Adenovirus detection in ground water URCM.
- Advantages: Rapid, can detect any type of microbe (specific); standards have been developed; new instruments available.
- Main Disadvantage detects viable and non-viable unless combined with culture.



The Risk to Human Health

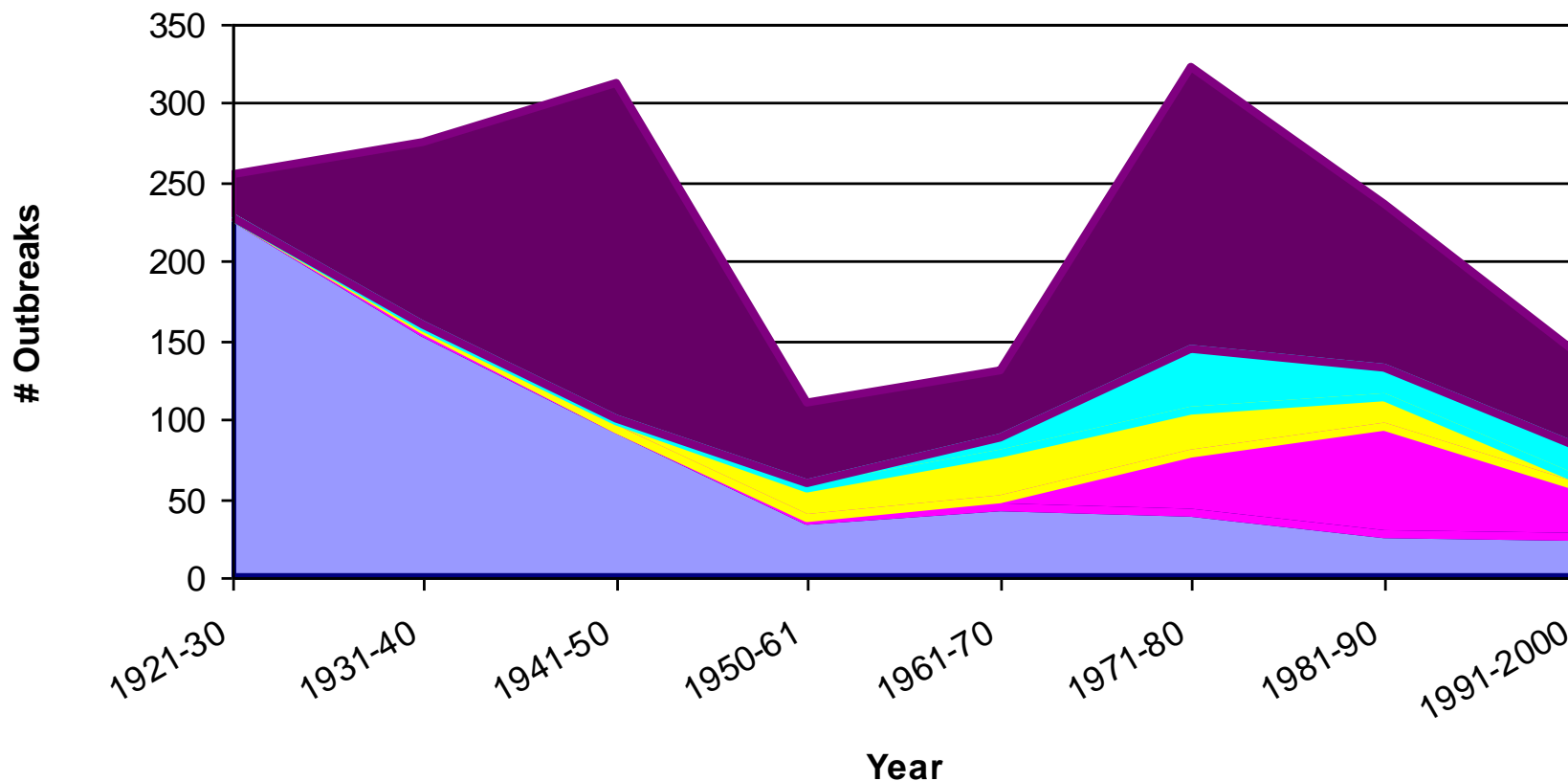
- ~19 Million waterborne illnesses /yr for community water systems in the US
 - 5.4 M illnesses from groundwater
 - 13 Million illnesses from surface water systems.
- (Reynolds et al. 2008)
- 12 Million cases/yr
(Colford et al. 2006)
 - 16 Million cases/yr
(Messner et al. 2006)



Waterborne Disease Outbreaks in the USA

Number of Waterborne-Disease Outbreaks Associated with Drinking Water, by Decade and Etiological Agent in the USA

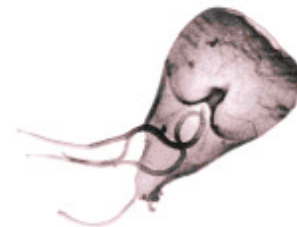
■ Bacteria ■ Parasitic ■ Viral ■ Chemical ■ AGI*



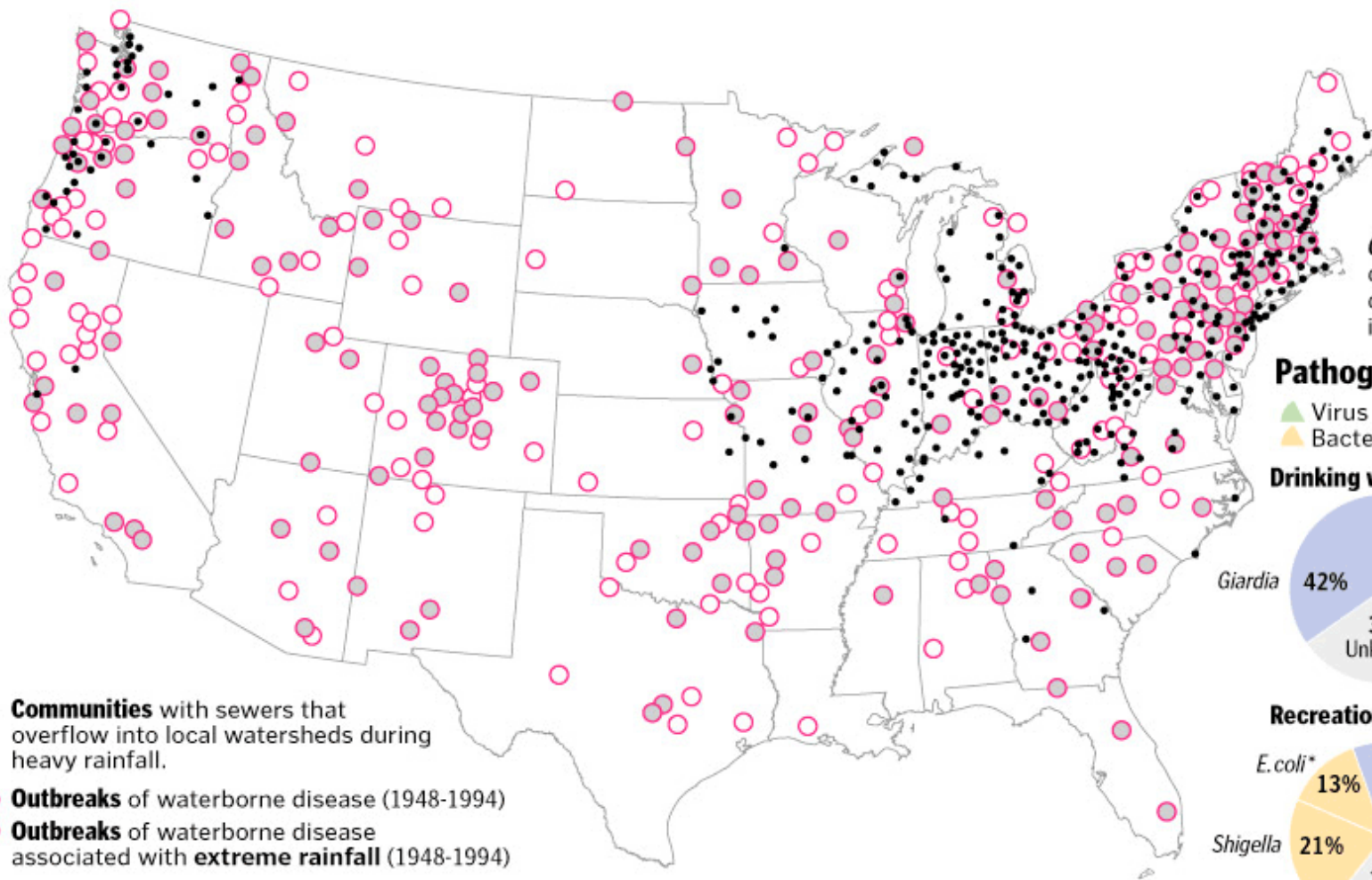
Risk of Disease Rises With Water Temperatures

By Kari Lydersen Washington Post Staff Writer

Monday, October 20, 2008



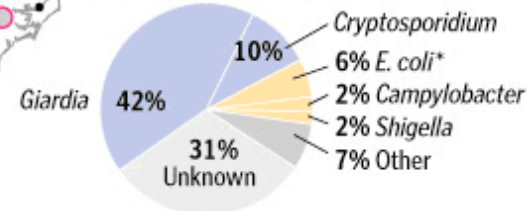
Giardia, found in sewage and contaminated streams and lakes, can cause severe gastrointestinal infections.



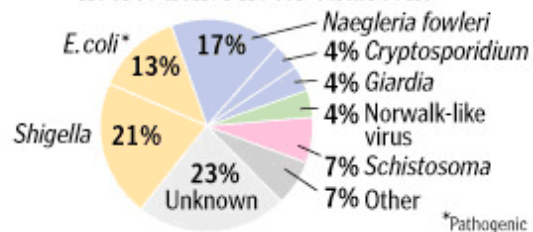
Pathogens (1985-2000)

- ▲ Virus
- ▲ Protozoan
- ▲ Bacterium
- ▲ Flatworm

Drinking water outbreaks



Recreational water outbreaks



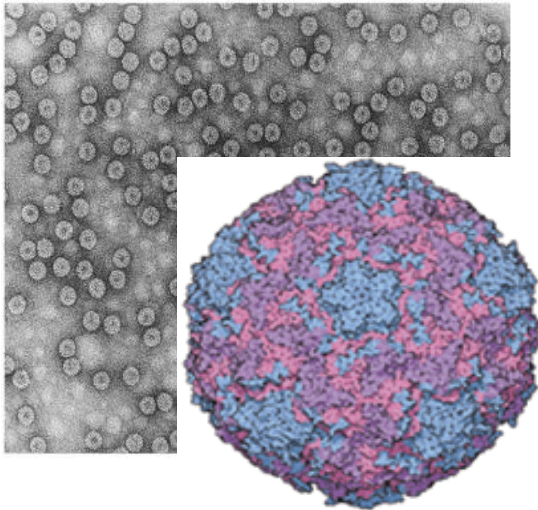
*Pathogenic

• **Communities** with sewers that overflow into local watersheds during heavy rainfall.

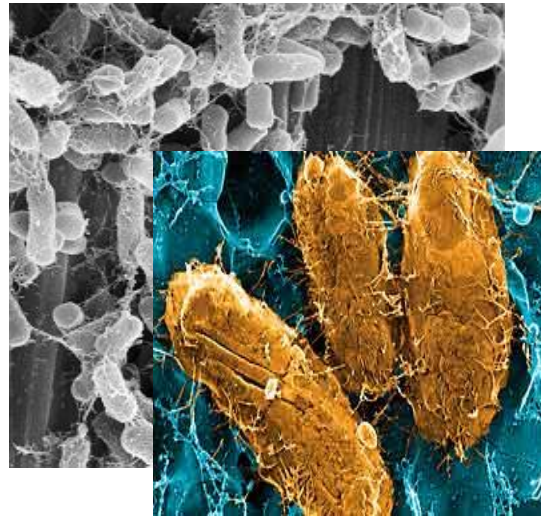
- **Outbreaks** of waterborne disease (1948-1994)
- **Outbreaks** of waterborne disease associated with **extreme rainfall** (1948-1994)

Types of Waterborne Pathogens

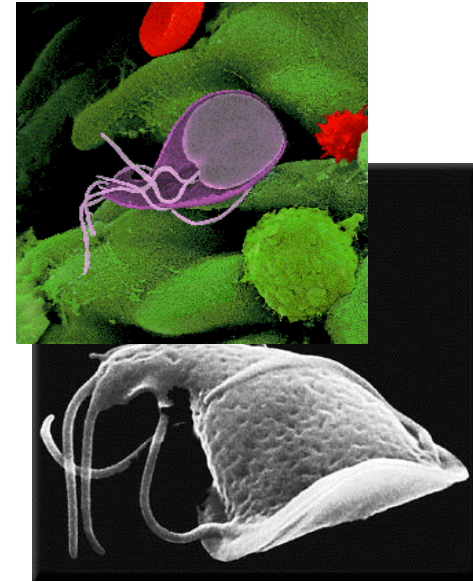
Viruses



Bacteria



Parasites

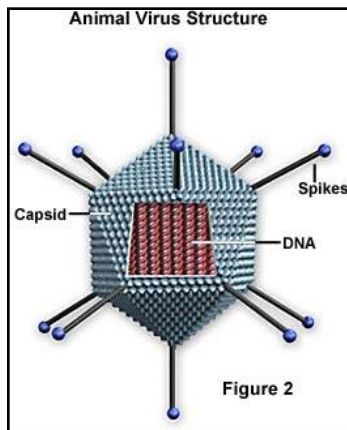


THE DISEASES: diarrhea, respiratory illness, liver damage, kidney failure, heart disease, cancer, nervous system disorders, ulcers, birth defects, death.

Predominant Waterborne Microorganisms

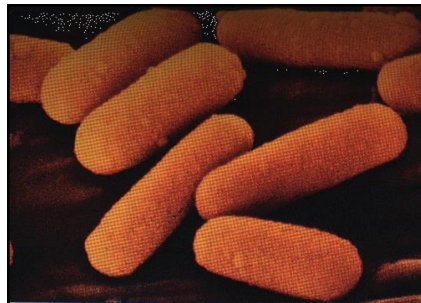
Viruses

- rotavirus
- adenovirus
- coxsackievirus
- echovirus
- calicivirus
- norovirus
- Hepatitis A and E



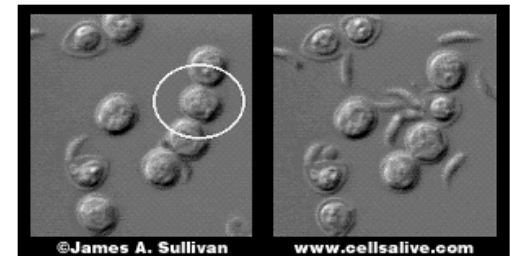
Bacteria

- *E.coli*
- *Salmonella* spp.
- *Shigella* spp.
- *Aeromonas hydrophila*
- *Campylobacter jejuni*
- *Helicobacter*
- *Mycobacteria*
- *Legionella*



Protozoa

- *Cryptosporidium parvum*
- *Cryptosporidium hominis*
- *Giardia lamblia*

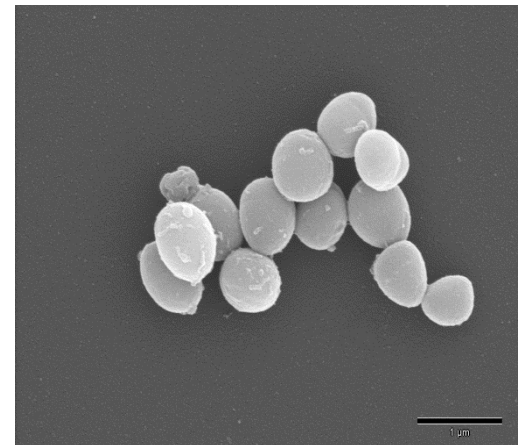
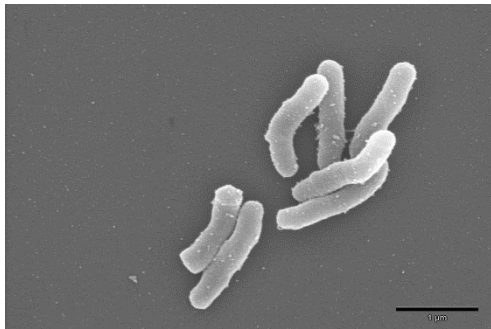


Ulcers From Drinking?

RAH RICHARDSON

Water, that is. Ulcers are caused by a bacterial infection, and in one region of Colombia, at least, the bug is in the water supply.

- ❑ The WHO has classified *H. pylori* as a Class I carcinogen because of the association of *H. pylori* and gastric malignancies.
- ❑ **German group ,Rolle-Kampczyk et al. (2004) found a significant correlation between well water contaminated with *H. pylori* detection by PCR and colonization status in humans.**
- ❑ Water supplies contaminated with fecal material may be a potential source of *H. pylori* transmission (Hulten *et al.*, 1996).



Waterborne Guillain Barré Investigation Update

August 5th, 2011 AZ

- a rare cluster of Guillain-Barré Syndrome (GBS) along the US-Mexico border near Yuma, AZ has been identified 8 more cases for a total of 24.

***Clostridium difficile* contamination of public tap water distribution system during a waterborne outbreak in Finland 2007 (KOTILA et al. Scandinavian Journal of Public Health, 2013; 0: 1–5)**

- 8000 people ill multiple etiological agents including 12 toxin producing *C. difficile* isolated from sewage contaminated drinking water in a large gastroenteritis outbreak town of Nokia, Finland.

Emerging Waterborne Disease Of the 21st Century

Low Infectivity

Environmentally
Robust

Zoonotic

Resistant to
Chlorination



**400,000 people ill
(50% of
the population)**

100 died

Cattle blamed

**Sewage blamed;
genetic
studies later
confirmed
sewage as one of the
causes.**



WILL CLINTON TESTIFY?
THE CAPITOL HILL GUNMAN

TIME

THE KILLER GERM

It's turning up
everywhere: in
your water, your
food, the pool.
How to protect
yourself from

E. COLI

NO POSTAGE
NECESSARY
IF MAILED
IN THE
UNITED STATES
POSTAGE
PAID
AT
NEW YORK, NY
PERMIT NO. 6000
NEW YORK, NY

Campylobacter and *E.coli* 0157 outbreak from manure contamination of groundwater

More US Waterborne disease Outbreaks occur In small communities Using Ground water.

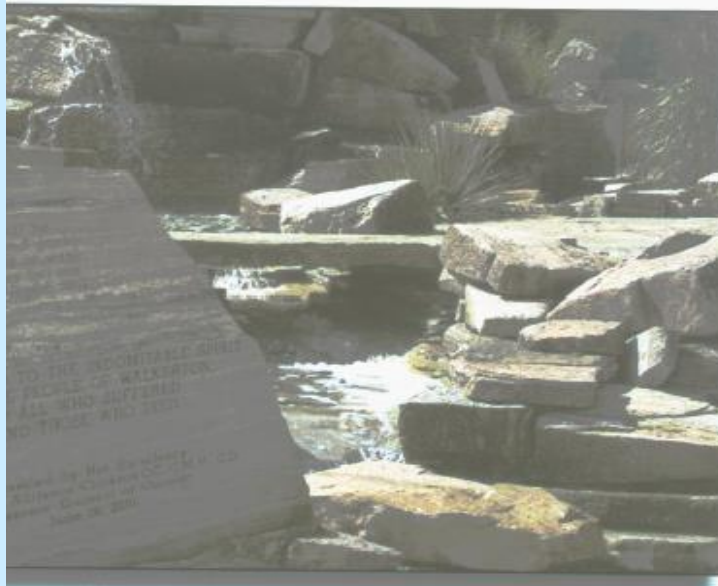
Safe Application of Animal Waste & Biosolids On land.

Protection of Ground water levels
Protection from septic tank Contamination.

Vol. 31, No. 6 June 2005

Walkerton — 5 years later Tragedy Could Have Been Prevented

by Steve E. Hrudehy and Richard Walker



In May 2000, several serious flaws in the Walkerton, Ont., municipal drinking water system aligned to permit a breakthrough of *E. coli* O157:H7 and *Campylobacter* bacteria, causing seven deaths and more than 2,300 cases of waterborne disease. These included 27 cases of hemolytic uremic syndrome, a serious kidney ailment with potential lifelong implications. Most of the cases of kidney disease were among children aged one to four. Other Walkerton residents have also reported enduring illness.

Ohio blames groundwater for Lake Erie island outbreak

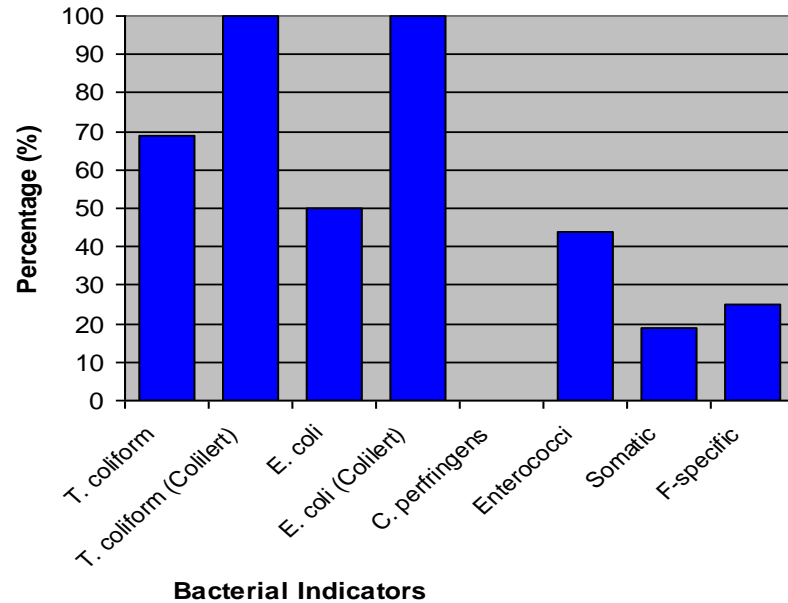
Tuesday, February 22, 2005

ASSOCIATED PRESS

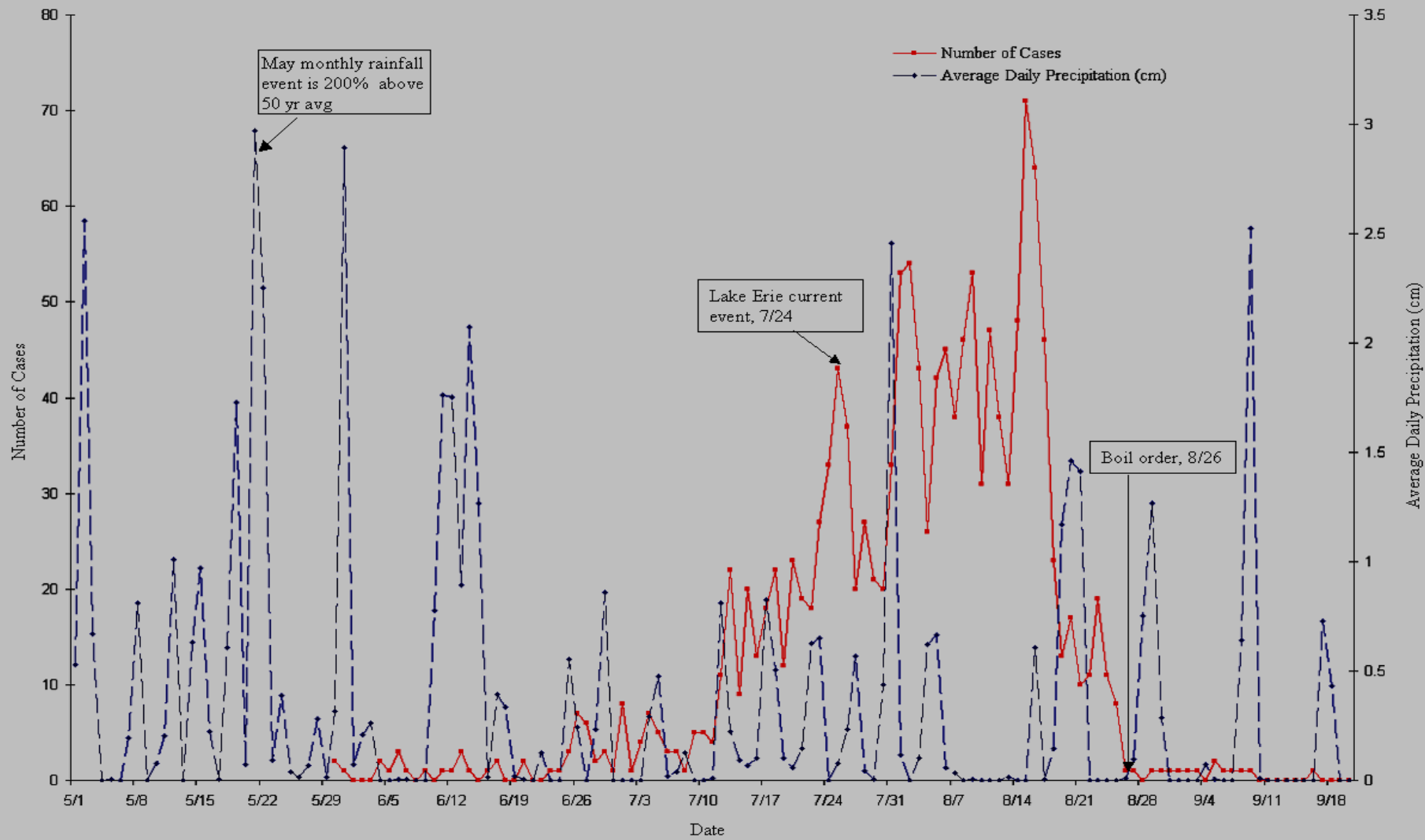
TOLEDO, Ohio -- Widespread groundwater contamination on a Lake Erie resort island was the likely source of illnesses that sickened hundreds last summer, the Ohio health department said Tuesday.

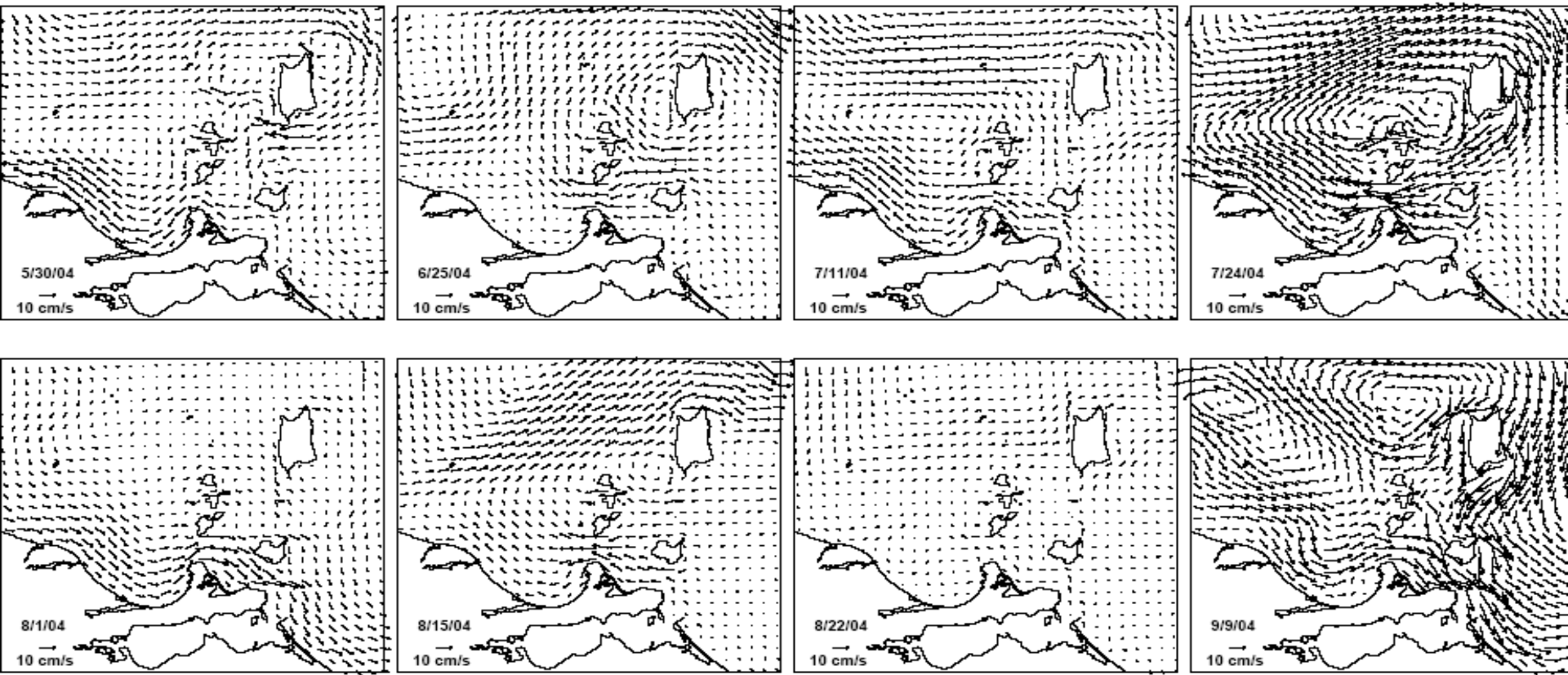
Several sources, including septic tanks, have tainted the South Bass Island's groundwater over a long period, and the contamination may have been worsened last summer because of a season of heavy rains, a health department report said.

The outbreak of gastrointestinal illness sickened about 1,400 tourists and residents, ending the tourist season early for many businesses.



MSU assisted with the investigation
Identified virus contamination and
potentially a new and emerging bacteria





South Bass Island
 Lake Erie
 Sources septic Tanks
 & sewage discharges
 Massive Ground Water
 and Surface Water
 Contamination



Arcobacter

- formerly classified as a *Campylobacter*
- aerotolerant, & are able to grow at 15 ° C
- higher prevalence than *Campylobacter* spp. in a S. African environmental & drinking water survey
- Diseases caused: enteritis, septicemia (blood poisoning) & colitis
- Emerging foodborne and waterborne pathogen

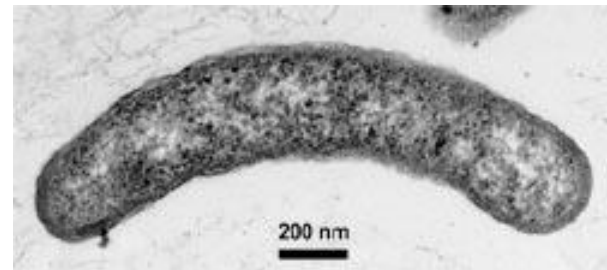
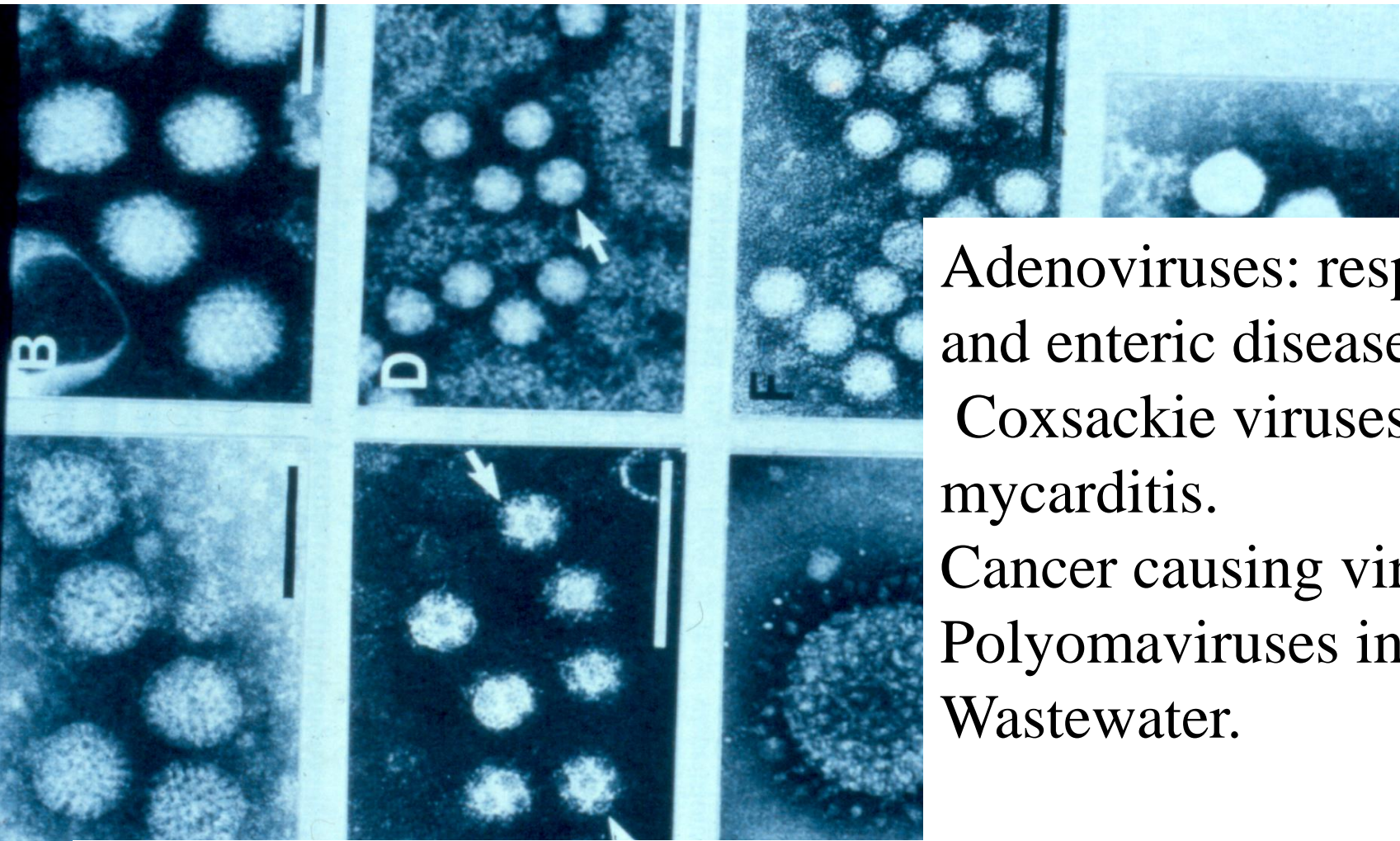


Photo courtesy: Craig Taylor & Carl Wirsén, WHOI



Adenoviruses: respiratory
and enteric disease

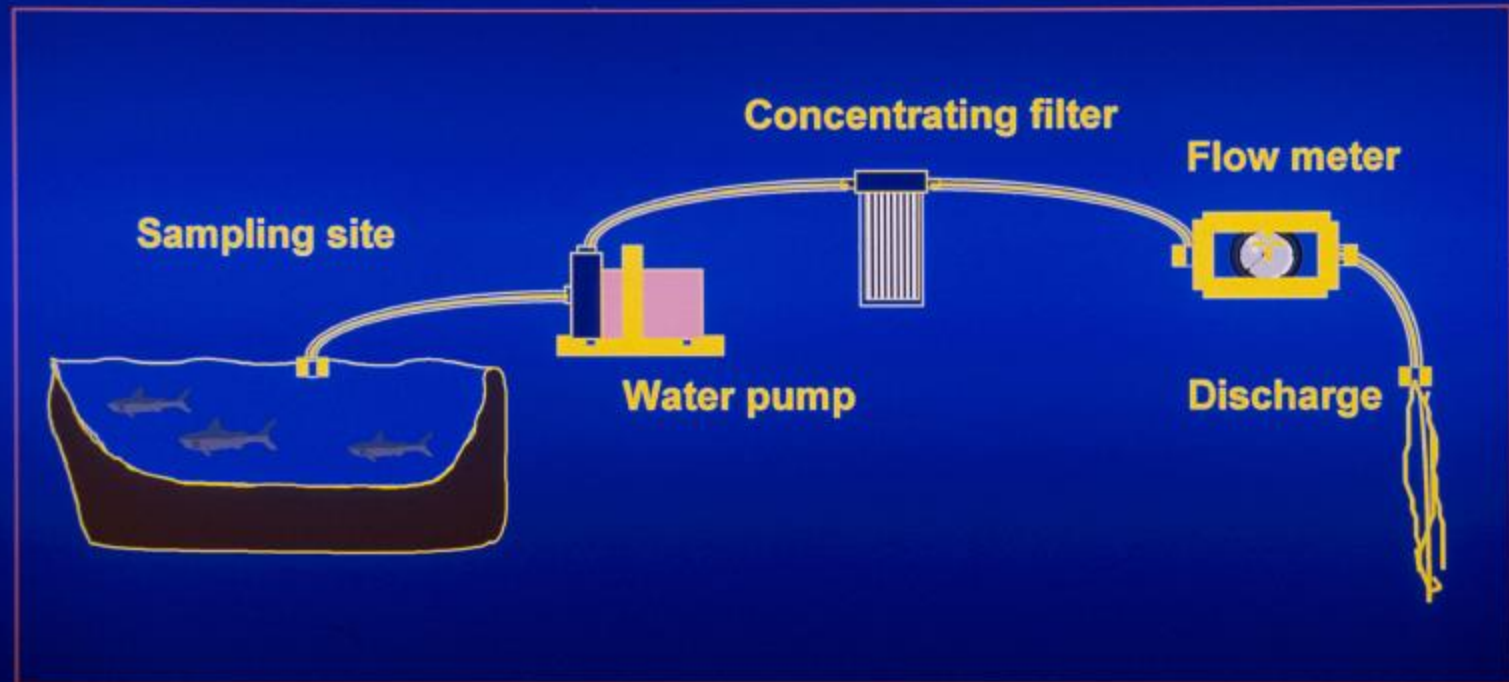
Coxsackie viruses caus
myocarditis.

Cancer causing viruses

Polyomaviruses in
Wastewater.

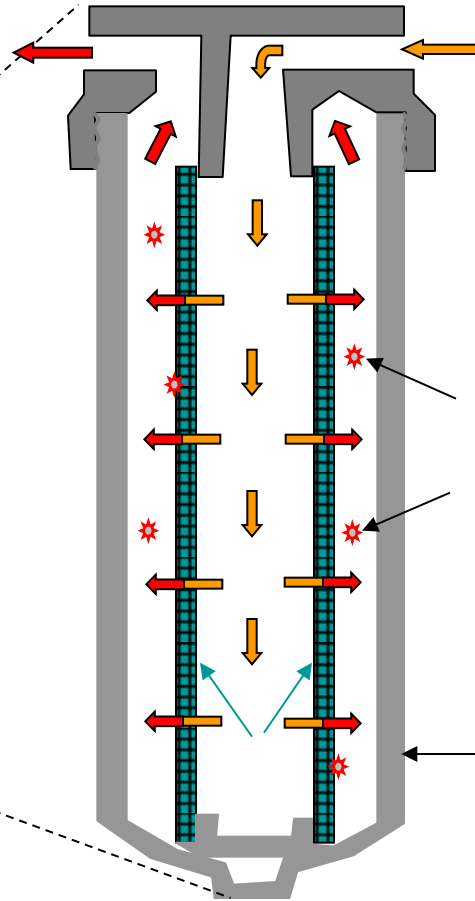
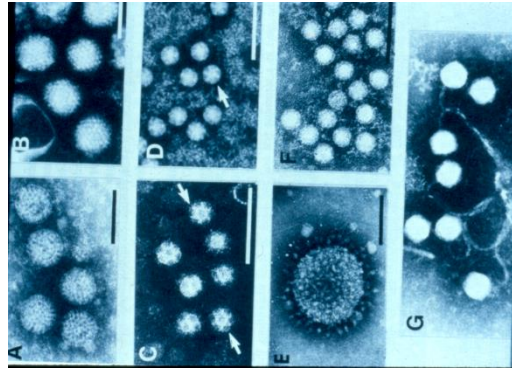
**VIRUSES ARE
BIO NANO PARTICLES**

Concentration of viruses from water



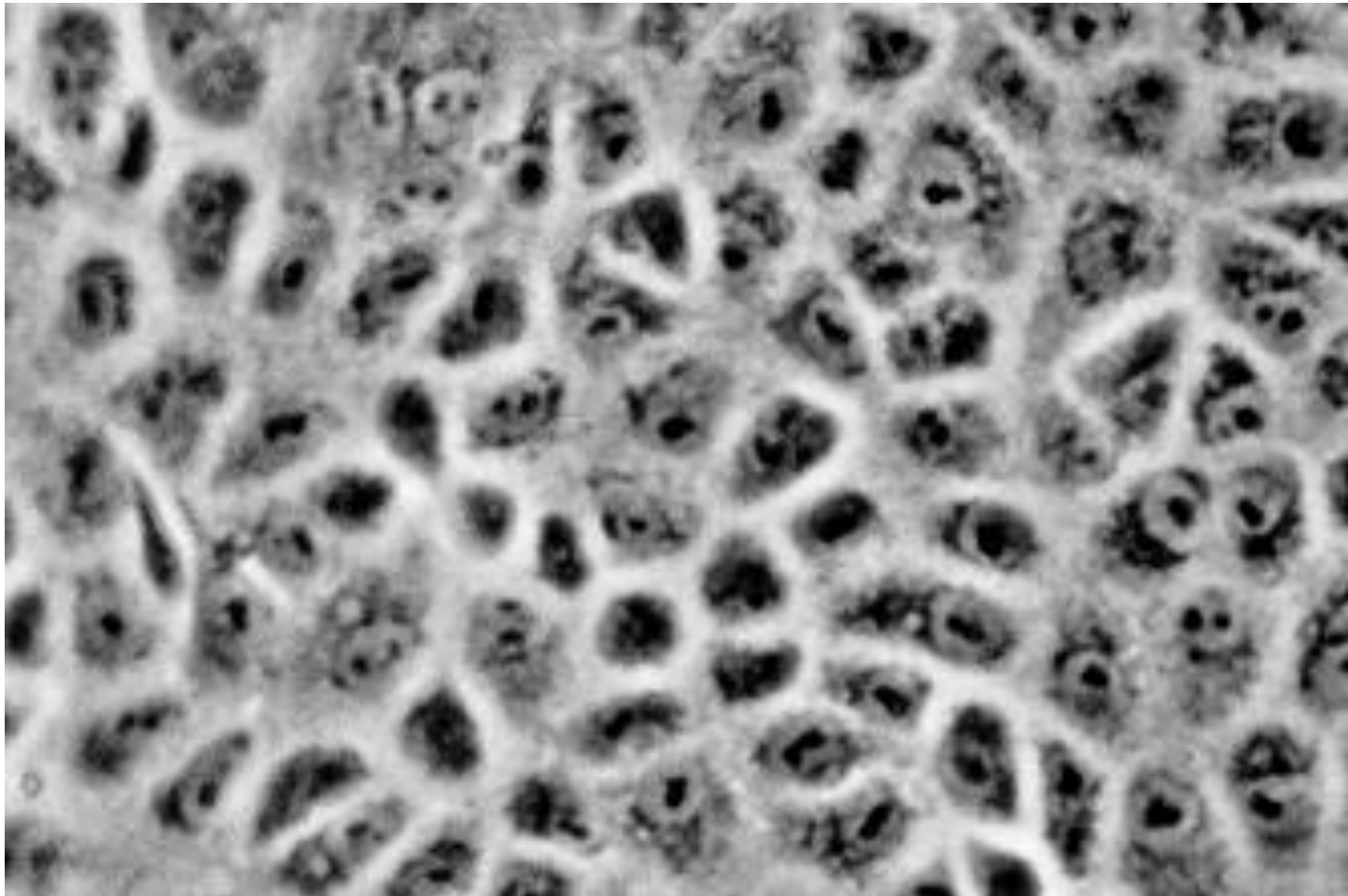
Emerging Microbial Contaminants

Determining VIRUS Risks

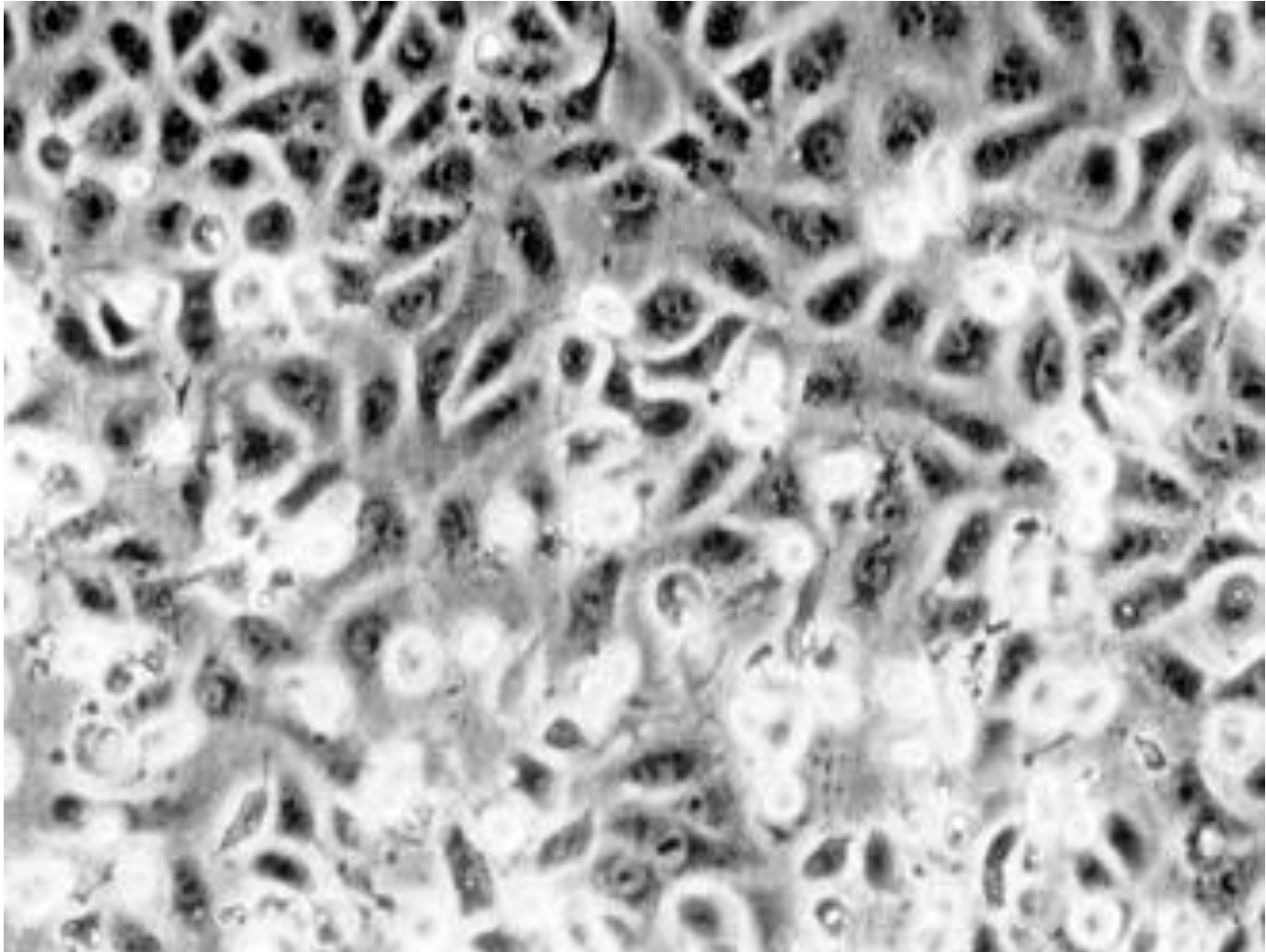


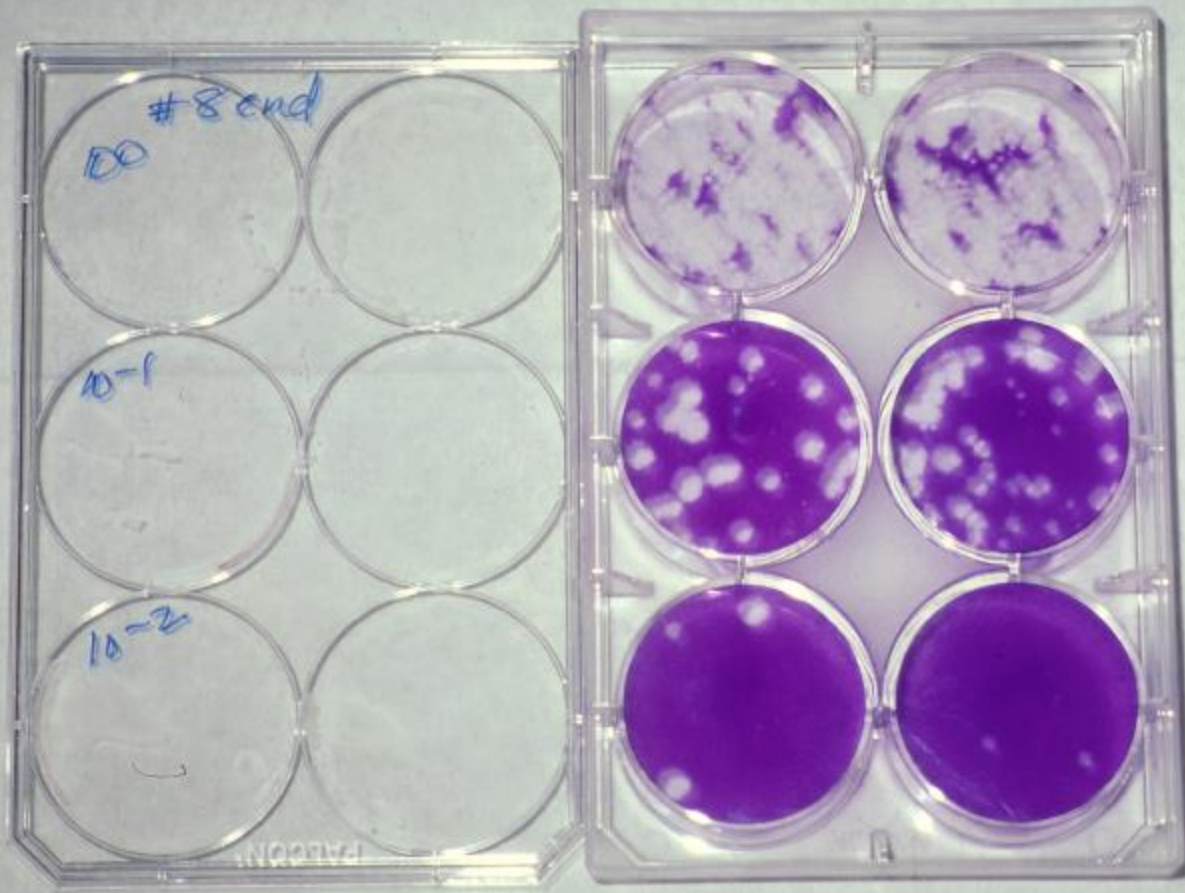


Noninfected monkey kidney cells



Monkey cells infected with poliovirus





Virus Occurrence

Occurrence of Human Adenoviruses at two recreational beaches of the Great Lakes

Xagorarakis et al., 2007, AEM, 73 (24), 7874-7881.

-Silver Beach up to 10^2 viruses/L

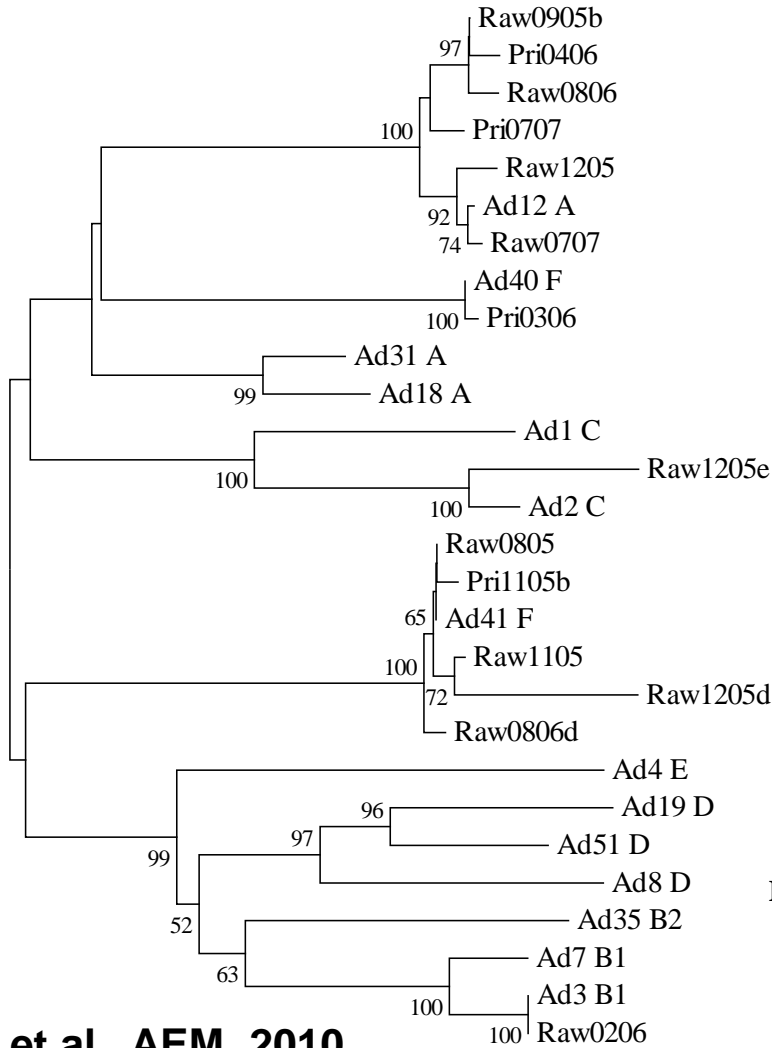
-Washington Beach up to 10^3 viruses/L

Quantitative Detection Of Human Adenoviruses In Waste Water, Surface Water, And CSO Discharges, In Michigan

Fong et al. 2010 AEM 76(3) 715-723

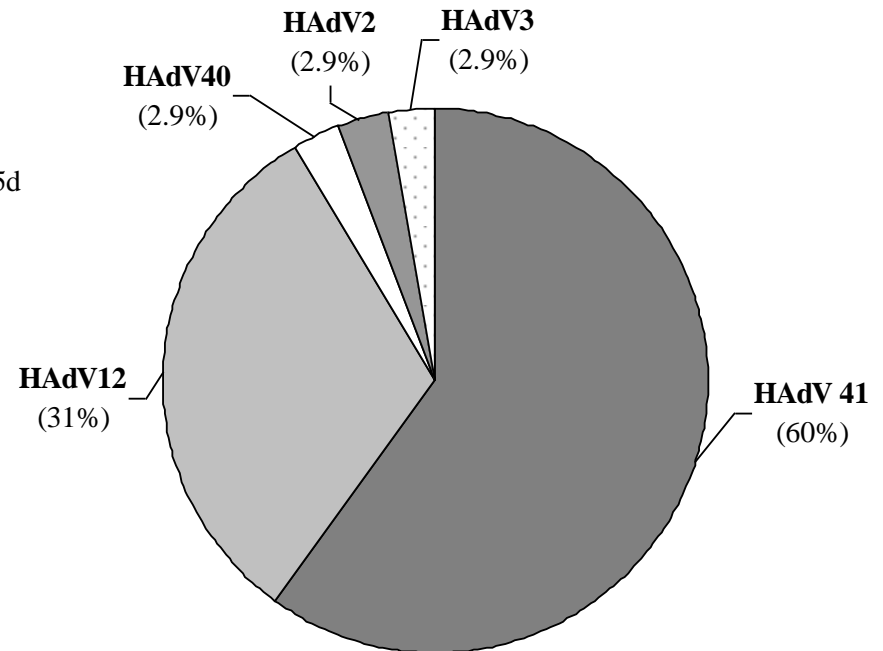
	qpcr /L	Averages
Raw	1152*	263-2817
Primary	1123*	53.7-4094
Secondary	20**	1.05-44.2
Tertiary	83**	13.5-428

Adenoviruses in Water

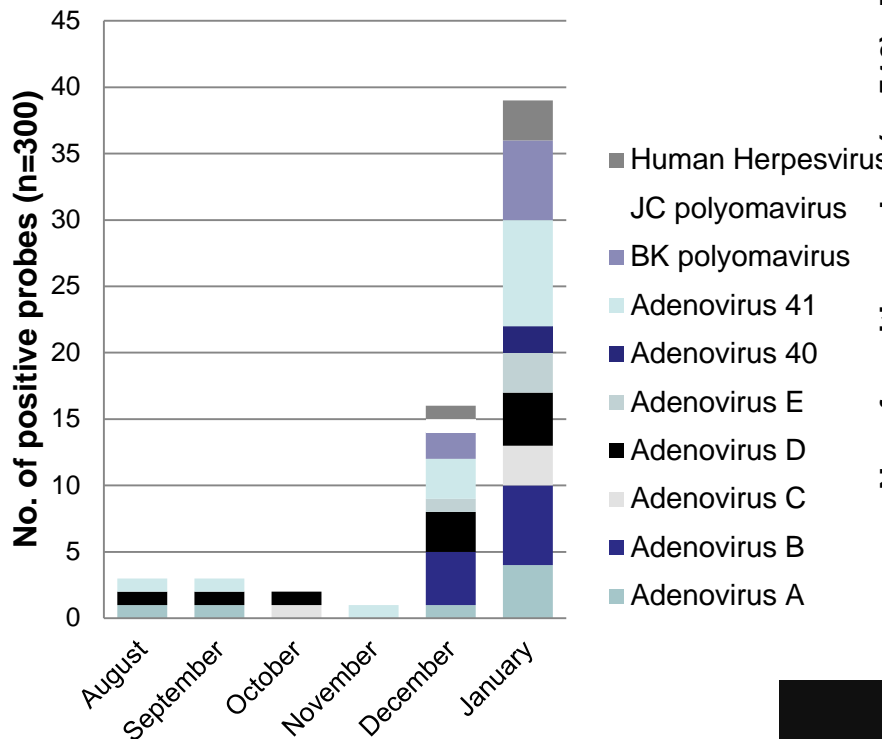


Fong et al., AEM, 2010

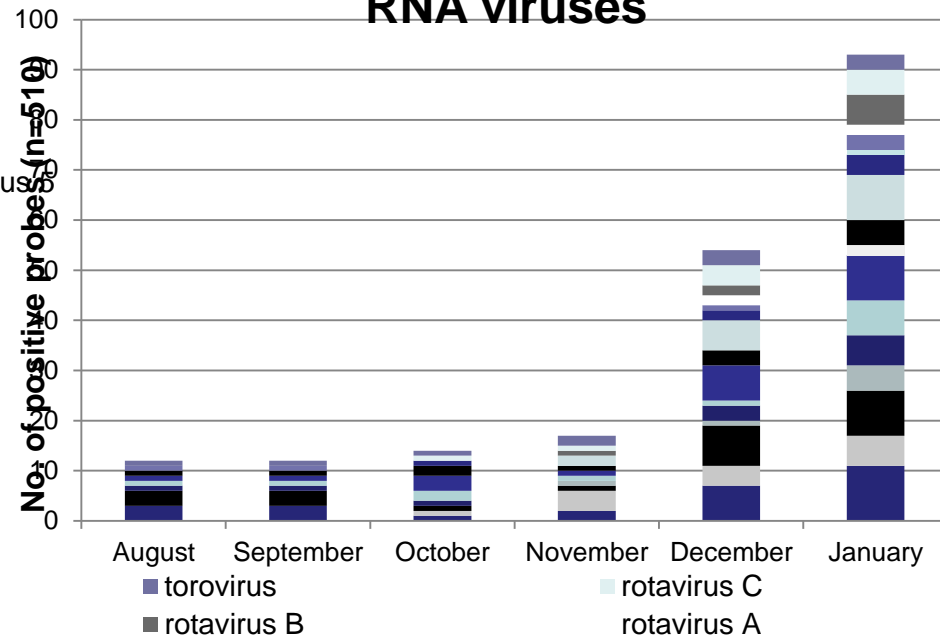
0.05



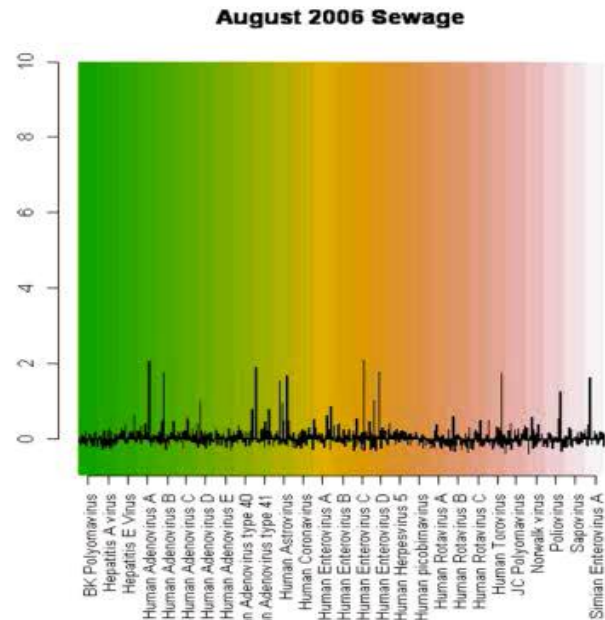
DNA viruses



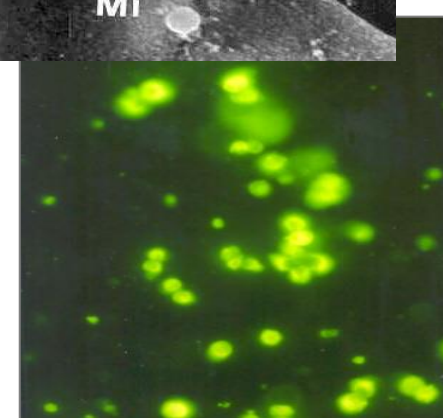
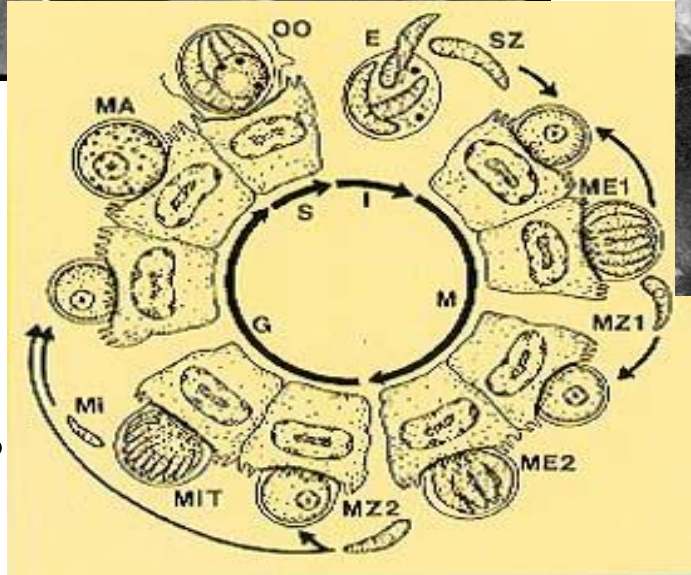
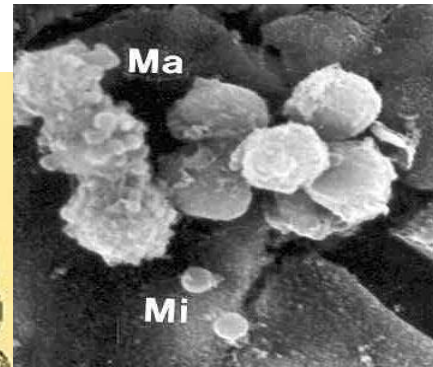
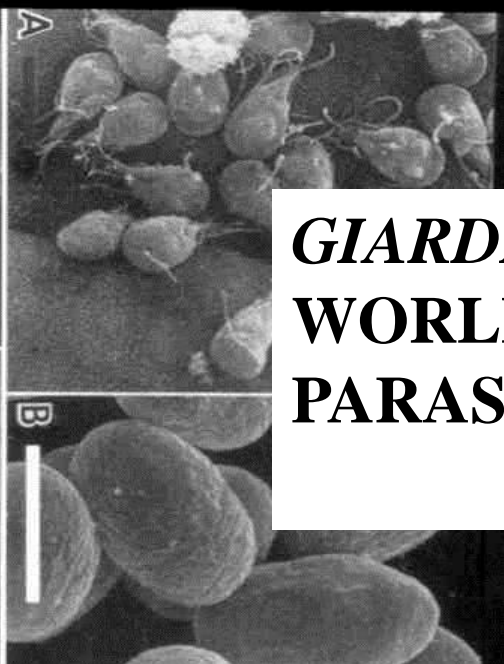
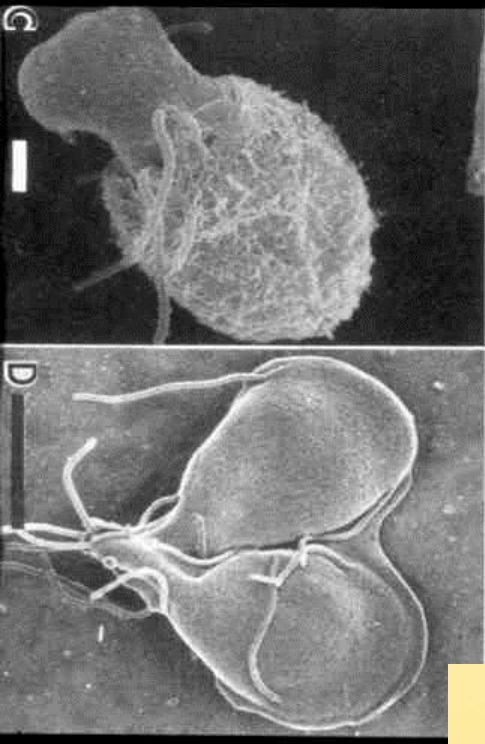
RNA viruses



Seasonal Enteric
Virus excretion
patterns in sewage
(Wong et al., 2008)



GIARDIA & CRYPTOSPORIDIUM
WORLD WIDE WATERBORNE
PARASITES OF CONCERN



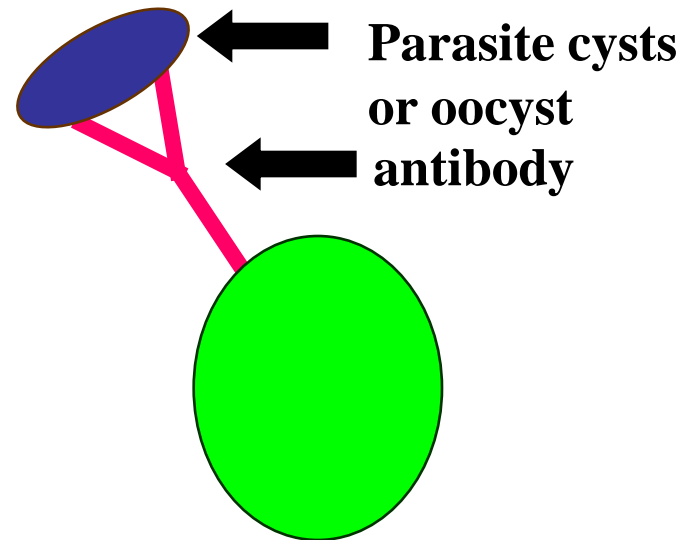
Hard to kill
Problem in
AIDs Patients

Parasite Concentration



Envirocheck Sampling Capsule

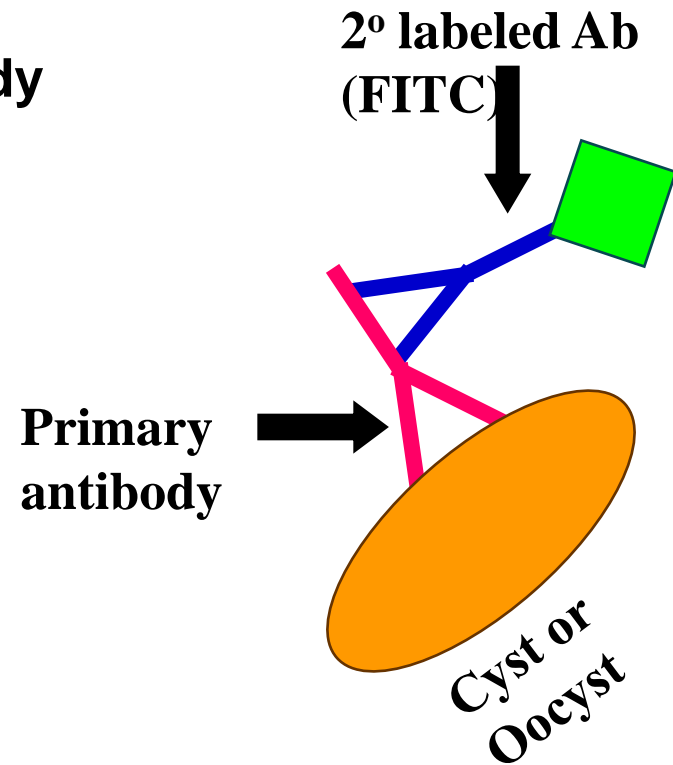
Purification by IMS



**Paramagnetic
microbead**

Detection by IF

- Detection method is based on an indirect immunofluorescent antibody (IFA) stain



Examination



Read slide under UV epifluorescent microscope
Presumptive



Cryptosporidium
4-6 μ m



Giardia
8-12 μ m

Confirm

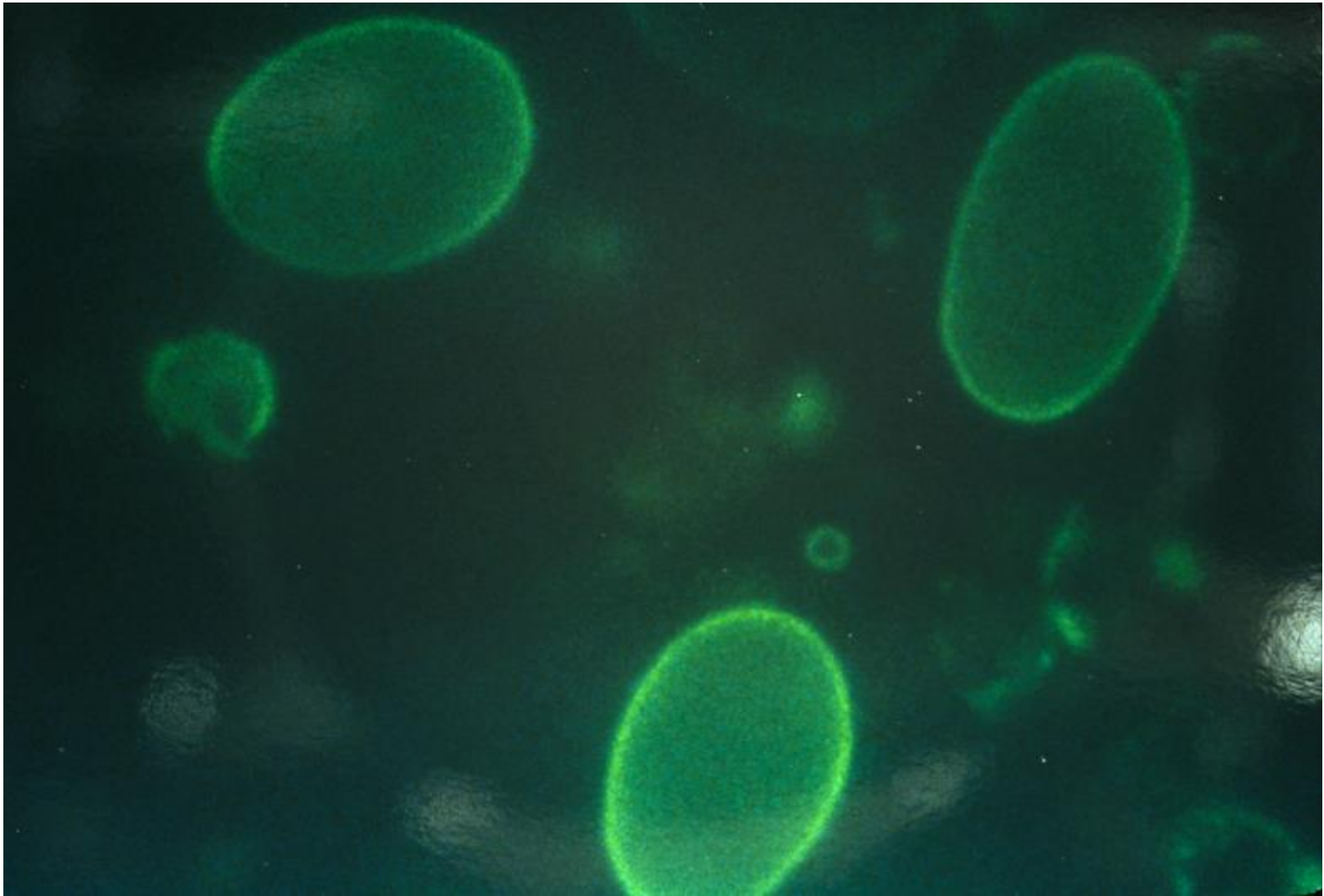
using Differential Interference Contrast

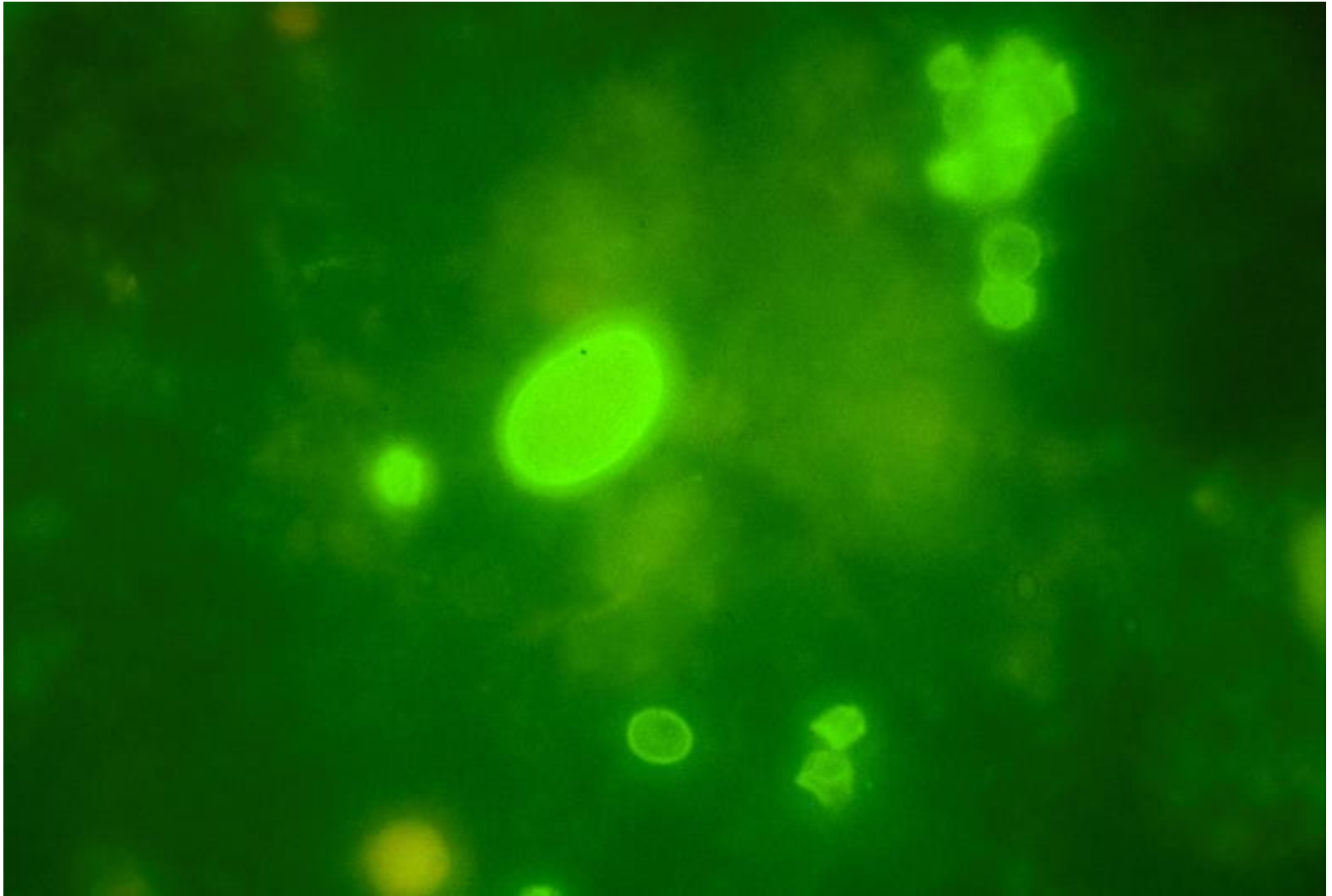


see up to four sporozoites

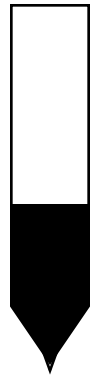


see nuclei,
axoneme &
median bodies

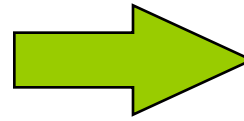




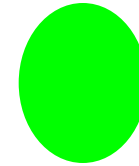
Cell Culture-*Cryptosporidium*



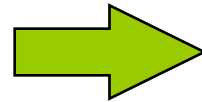
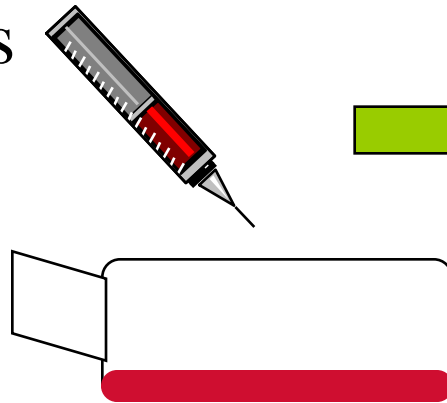
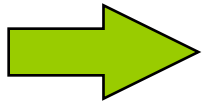
Take purified sample
expose to 10% bleach for
10 min to inactivate
viruses and bacteria,
algae, fungi



Wash and perform
excystation



Inoculate sample onto
HCT-8 cells

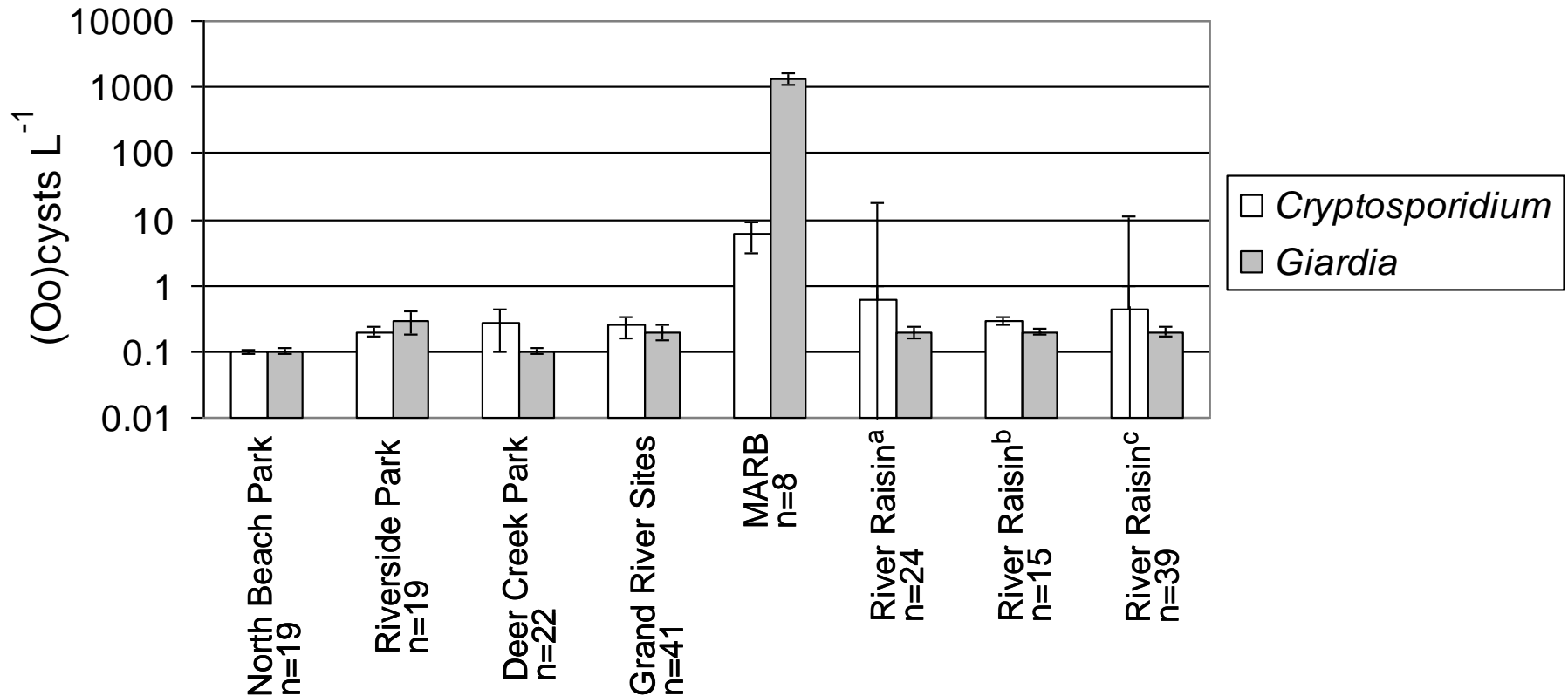


Examine cells for
evidence of infection and
growth of *Cryptosporidium*
life stages

- microscope (bright field/IFA)
- ELISA
- PCR

Sampling Results Summary

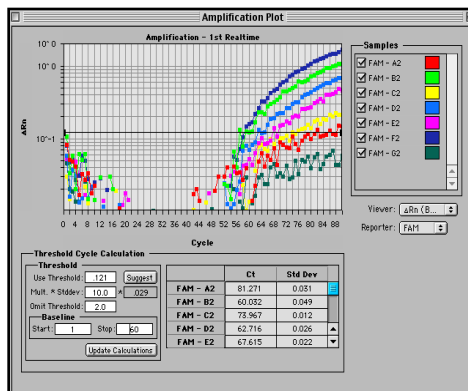
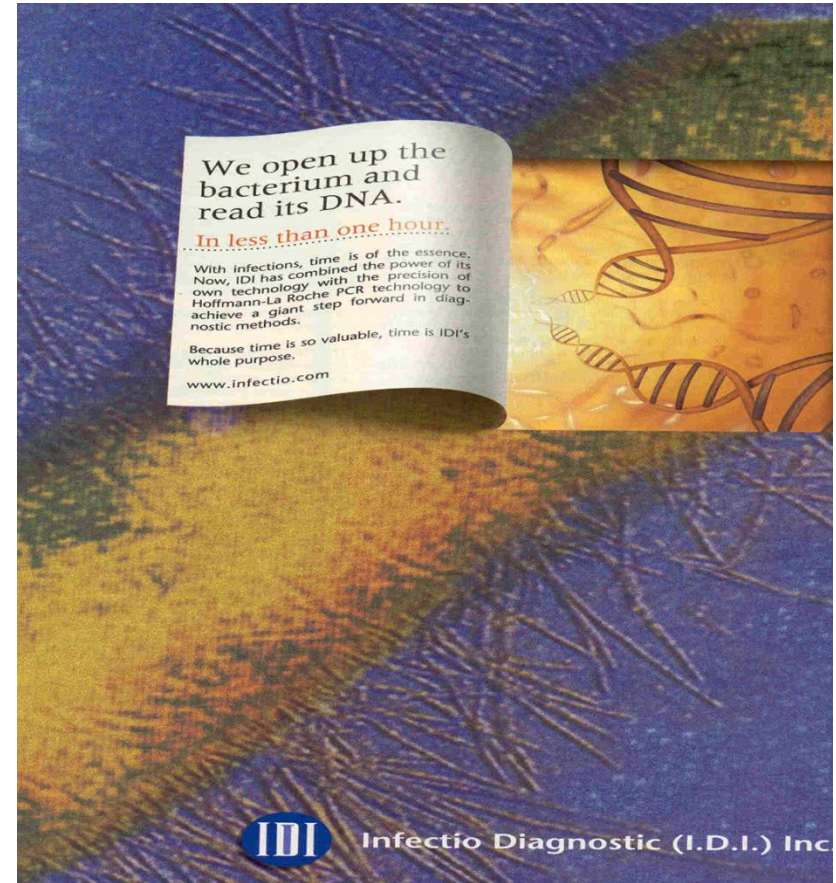
Parasite occurrence: Geometric mean



^a Sites upstream of CSO outfalls ^b Sites downstream of CSO outfalls
^c Total River Raisin sites

Source Tracking

- Methods are now available to determine the source of fecal contamination
- Question: is the fecal contamination from human sources?



Sources of *E.coli* and Pathogens

Septic systems



Waste water/Sewage treatment



Animal farming operations



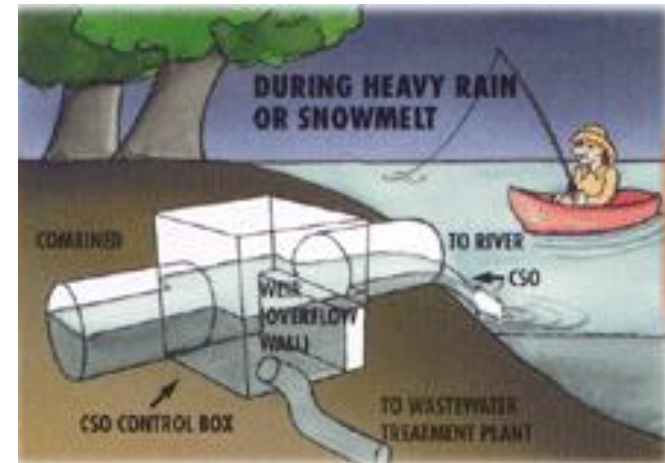
Agricultural run-off



Wildlife



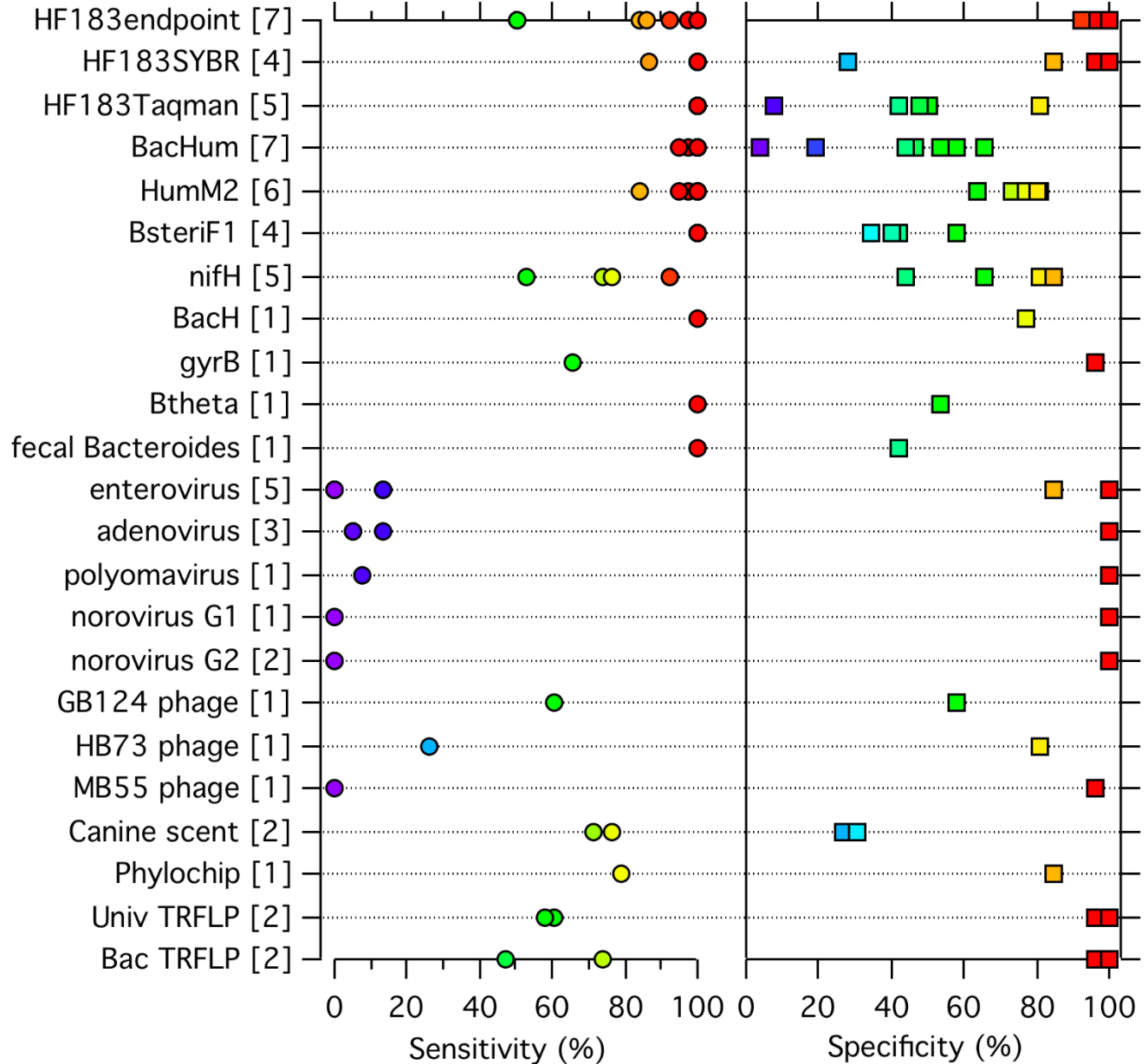
Combined Sewer Overflow



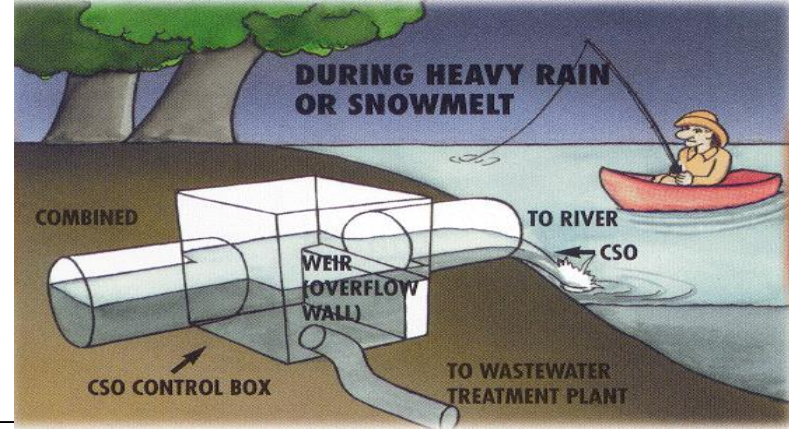
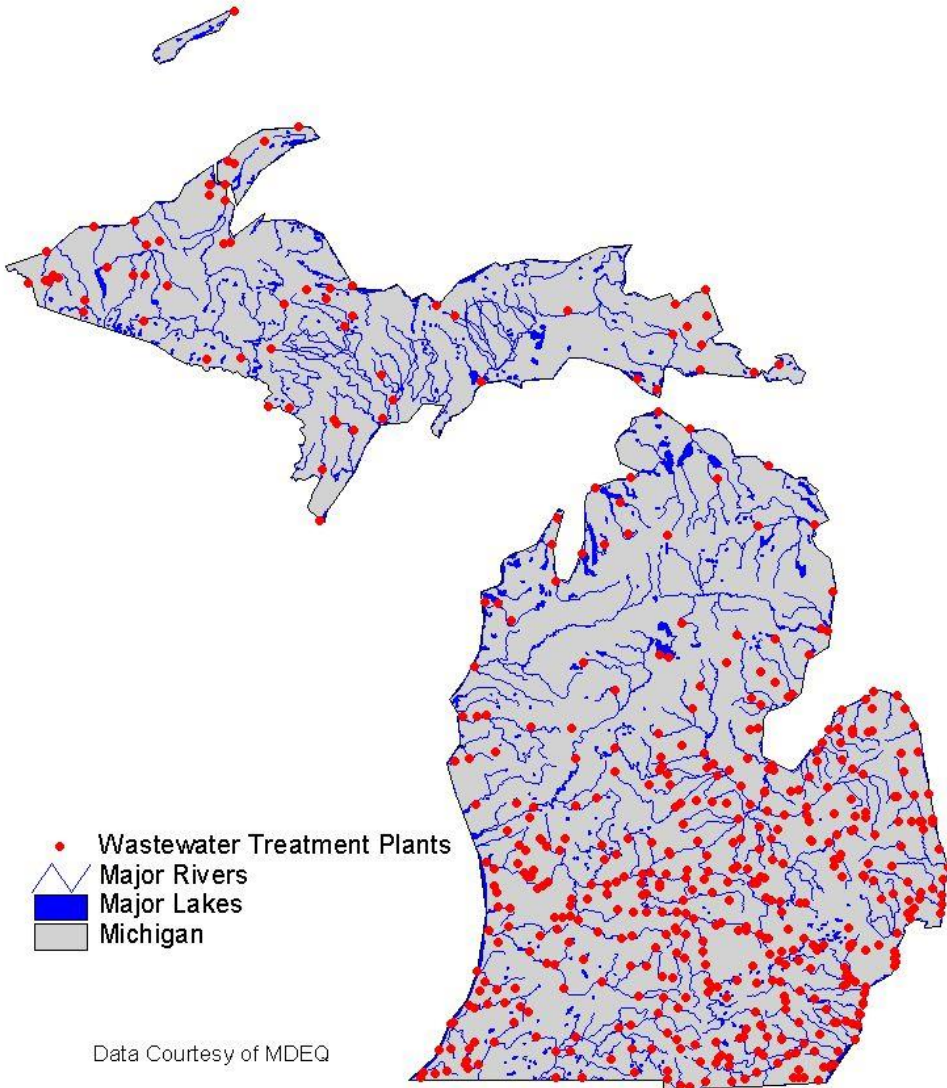
Human Assays (Layton et al. 2012)

BtH
12 samples
from Humans
26 samples
from non-
humans

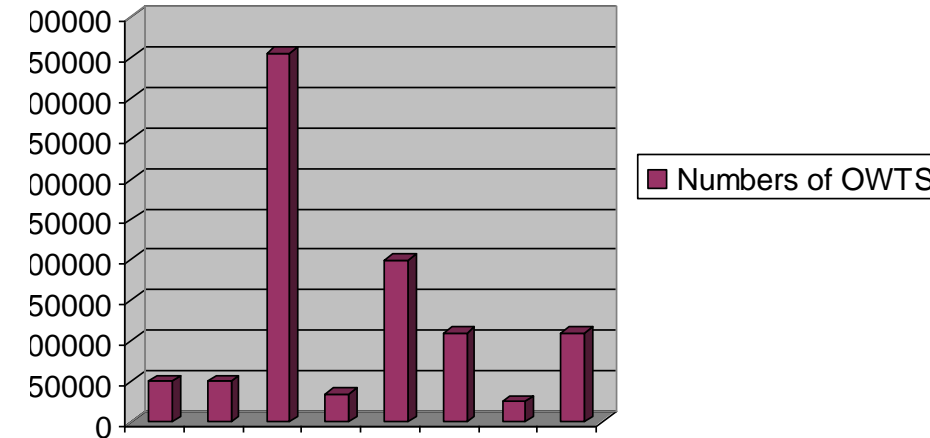
DNQ as (-)
92% and 96%
Sensitivity and
Specificity



Wastewater Treatment Plants vs. Major Rivers and Lakes



On site Wastewater Systems in the Great Lakes



~1.7 million septic tanks in Michigan

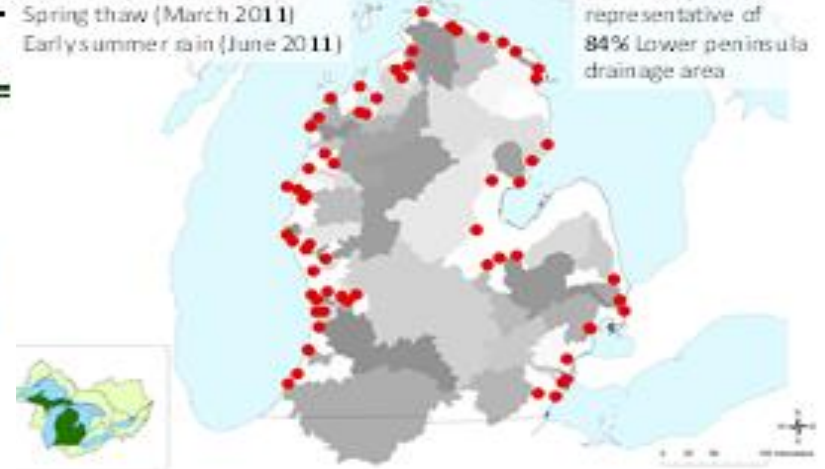
The distribution of the human sewage marker *Bacteroides*

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STUDY LOCATION

- Baseflow (October 2010)
- Spring thaw (March 2011)
- Early summer rain (June 2011)

64 River systems representative of 84% Lower peninsula drainage area.



RESULTS: SPATIAL-*B. THETA*

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B. theta concentration range (Cells/100ml)



Northwest

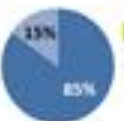


Northeast

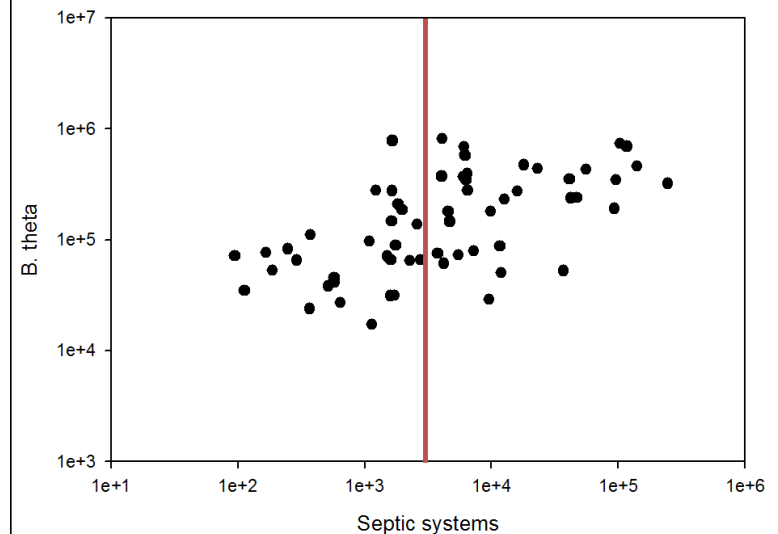


Southwest

■ Detections
□ Non-detections



Southeast



Water Quality Monitoring Needs

- ❖ **Understand the sources and level of fecal pollution in our watersheds and groundwater**
- ❖ **Develop strategic pathogen monitoring programs**
- ❖ **Use new diagnostic tools**
- ❖ **Develop training programs for use of qPCR in water laboratories**



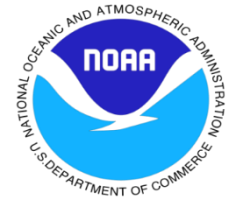
Acknowledgements

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CWS
Center for Water Sciences





THANK YOU

